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Ecological change in shallow lakes through antifoulant biocide contamination

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I, Daniel John Hoare confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

This study sought to determine whether tributyltin (TBT), a toxic environmental contaminant now banned from use in antifoulant paints, could have contributed to ecological degradation in shallow lakes. Anthropogenic stresses have often led to changes in ecological structure and functioning within such waterbodies, with catastrophic loss of submerged macrophytes a common phenomenon. An area that has experienced intense TBT contamination and widespread macrophyte loss, is the Broads, a popular inland navigable waterway in E. England.

Development of an online SPE-LC-MS^n analytical method enabled identification and quantification of contemporary organic antifoul biocides in water and sediment samples. This contemporary analysis improved understanding of the transport mechanisms that would have been responsible for dilution and dispersion of TBT. Within the River Bure study area, a distinct antifoul biocide contamination gradient was observed, that related to the level and type of boating activity. Most significantly, biocide transportation has led to areas not directly exposed to boating activity, but in hydrological connection, to become contaminated. The recent ecological histories of contaminated lakes was reconstucted using multi-proxy palaeoecological analytical techniques on cores collected using a new wide-diameter corer. Data from the radiometrically-dated cores indicated that at least twenty years of continuous TBT pollution occurred in the Broads, against a backgound of eutrophication. The pre-TBT period was characterised by presence of macrophyte remains with abundant plant-associated diatoms, cladocera and invertebrates, which switched to predominantly planktonic assemblages after initial detection of TBT. Environmental concentrations of TBT present during its active usage in antifoulant paints, would have adversely affected functionally important aquatic organisms, as indicated by ecotoxicological test data.

The spatio-temporal assessment of contamination, combined with a palaeoecological approach, has been successful in reconstructing relative toxicant exposure and patterns of ecological change in the Broads. This methodology could be applied to the study of other persistent pollutants.

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TABLE OF CONTENTS

CHAPTER 3 – ORGANIC BIOCIDE ANALYTICAL METHOD DEVELOPMENT

CHAPTER 4 – CONTAMINATION OF ORGANIC ANTIFOUL BIOCIDES ALONG THE RIVER BURE WATERWAY

CHAPTER 5 – ORGANOTIN SURFACE SEDIMENT CONTAMINATION

CHAPTER 6 – PALAEOLIMNOLOGY OF TBT EXPOSED BROADS

CHAPTER 7 – SUMMARY AND CONCLUSIONS

LIST OF FIGURES

LIST OF TABLES

GLOSSARY OF ACRONYMS AND ABBREVIATIONS

ACE	automated cartridge exchange
ACP	Advisory Committee on Pesticides
AFP	antifoul paint
APCI	atmospheric pressure chemical ionisation
CEFAS	Centre for the Environment, Fisheries and Aquaculture Sciences
C/N	carbon to nitrogen ratio
CRS	constant rate of supply
DBT	dibutyltin
DCA	detrended correspondence analysis
DDT	dichloro-diphenyl-trichloroethane
d/s	downstream
EC	end capped
ECRC	Environmental Change Research Centre
EDC	endocrine disrupting compound
FIA	flow injection analysis
FPD	flame photometric detection
GC	gas chromatography
HPLC	high performance liquid chromatography
HSE	Health and Safety Executive
ID	internal diameter
IS	internal standard
K_d	**distribution coefficient**
K_{oc}	organic carbon adsorption **coefficient**
K_{ow}	octanol-water partition coefficient
LC	liquid chromatography
LC_{50}	lethal concentration (upon 50% of the test population)
LOD	limit of detection
LOEC	lowest observed effect concentration
LOI	loss on ignition
LOQ	limit of quantification
MAFF	Ministry of Agriculture, Fisheries and Food
MASE	microwave-assisted solvent extraction
MBT	monobutyltin
ME	matrix effect
MRM	multiple reaction monitoring
MS	mass spectrometry
NaOH-P	sodium hydroxide extracted phosphorus
NERC	Natural Environment Research Coucil
OM	organic matter
OS	Ordnance Survey
PAH	polyaromatic hydrocarbon
PCA	principle components analysis
PCB	polychloro-biphenyl
QC	quality control
RDA	redundancy analysis
RSD	residual standard deviation
RT	retention time
SD	standard deviation
S/N	signal to noise ratio
SPE	solid phase extraction
TBT	tributlytin
TP	total phosphorus
TSS	total suspended solids
UCL	University College London
u/s	upstream
UV-DAD	ultra-violet diode array detector
VDPB	Vienna Peedee belemnite
WWTW	wastewater treatment works

CHAPTER 1 - INTRODUCTION

1.1 General introduction

During the past few centuries a dramatic decline in the chemical and ecological quality of freshwater resources has been observed across the globe (OECD 1982;UNEP 1999). Especially vulnerable are shallow lakes (less than 3 m average depth), that are generally situated in fertile lowland areas, formed as post-glacial or floodplain features, or through human activities (Moss, Madgwick, and Phillips 1996a). High human population densities and intensive landscape management often surround such lakes (Scheffer 1998) and increased pressure on these water bodies has led to loss of biodiversity (De Nie 1987;Ricciardi and Rasmussen 1999); collapse of economically important fisheries (Bachmann, Hoyer, and Canfield 2004); increased water treatment costs and reductions in amenity value (National Research Council (U.S.) 1992). Recognition of factors responsible for such resource degradation is key to determining successful remediation and future management strategies of these culturally and ecologically important water bodies. However, identification and separation of the human-induced stresses upon lake ecosystems is often difficult once the degradation has already occurred.

A particular feature of shallow lakes is that they commonly exhibit either macrophyte dominance with clear water conditions, or phytoplankton dominance with turbid water conditions, accompanied by minimal or no macrophyte growth (Blindow *et al.* 1993). Both states can occur within a broad range of overlapping nutrient concentrations (Jeppesen *et al.* 1991), with the distinct ecological states being described as alternative equilibria (Scheffer *et al.* 1993). The human-induced loss of aquatic vegetation from shallow lakes is widely regarded as a decline in ecological quality, with active environmental management required to reverse the process (Moss *et al.* 1996a;Carpenter, Ludwig, and Brock 1999).

There is a lack of understanding in the role of toxic contaminants as environmental stressors in the degradation of shallow lakes, as most affected lakes have become degraded prior to the instigation of detailed environmental monitoring. Indeed the presence of such pollutants may remain undetected or given little attention as causal factors. This problem is worsened by the wide range of substances likely to contaminate shallow lakes, which includes heavy metals, polycyclic aromatic

hydrocarbons, pesticides and a wide variety of other synthesised chemicals. Ecotoxicological research has provided valuable insights into how freshwater ecosystems respond to such stressors, through experimental manipulations with toxic substances (Jak, Maas, and Scholten 1998c;Traas *et al.* 2004;Wendt-Rasch *et al.* 2004). However, in "real world" environmental situations where ecological change has already happened, palaeolimnological studies have been one of the few tools available with which to investigate toxicant stressors as potential agents in the observed decline of lake ecosystems (Stansfield, Moss, and Irvine 1989;Miskimmin, Leavitt, and Schindler 1995;Ilyashuk, Ilyashuk, and Dauvalter 2003;Paterson *et al.* 2003;Sayer *et al.* 2006). Similarly, environmental gradients of known pollutants have been used to demonstrate the spatial variability of impacts of toxic substances upon aquatic biota (Dickman, Yang, and Brindle 1990;Burton, Rundle, and Jones 2001). Field-based research has also demonstrated how the presence of a toxic substance can have radical effects upon the overall structure and function of an otherwise "dynamically" stable aquatic ecosytem (Mason *et al.* 2003).

A direct route for toxic substances to enter the aquatic environment arises where there are boats and ships which have their hulls coated with biocidal antifoulant paint. These formulations are designed to protect submerged surfaces from the nuisance colonisation of bacteria, algae and molluscs, through the constant delivery of toxic concentrations of biocides from the surface of the painted area. Copper oxides have formed the basic biocidal protection for many decades, with other toxic compounds added to increase the efficacy of the paint formulation against copper-resistant organisms. The organo-metallic compound tributyltin (TBT) has been the most successful "booster" biocide used to date, due to its potent toxicity to a wide range of marine and freshwater fouling organisms, especially mollusca (Maguire 1987). However, due to the persistence of this compound in the aquatic environment, areas with high densities of vessels coated with TBT-containing paint were found to be highly contaminated with TBT in water, sediment and biotic compartments (Maguire *et al.* 1982;Maguire and Tkacz 1985;Chau 1986;Valkirs *et al.* 1986;Waldock, Thain, and Waite 1987;Cleary and Stebbing 1987b). The negative impacts of TBT contamination was first noticed around important shellfish production areas (Alzieu 1998b). Further environmental research identified the harm that TBT was causing to other non-target biological communities (Laughlin, Nordlund, and Linden 1984;Cardwell and Sheldon 1986;Alzieu *et al.* 1986), which eventually led to a ban on it's usage on small vessels under 25 m in many countries

in the late 1980's (Champ 2000). Given this history of TBT pollution the compound has been described as the most acutely toxic chemical ever deliberately introduced to water (Hoch 2001). The less environmentally persistent replacement antifoulant biocides most commonly used since then have included the organic herbicides diuron and Irgarol 1051. These organic booster biocides have however been found to subsequently contaminate surface waters and sediment (Voulvoulis, Scrimshaw, and Lester 2000;Thomas, McHugh, and Waldock 2002;Comber, Gardner, and Boxall 2002;Hall, Killen, and Gardinali 2004), with often negative biological consequences (Nyström *et al.* 2002a;Balcomb, Hoberg, and Giddings 2003).

Antifoulant biocides by their very design are known to be toxic to freshwater organisms. TBT contamination within freshwaters is relatively under researched compared to that found in the marine environment, but, nevertheless, toxic effects on non-target freshwater biota have been widely demonstrated (Maguire 1991;Day *et al.* 1998;Loganathan *et al.* 2001;Bartlett *et al.* 2005;Leung *et al.* 2006). The spatial distribution of antifoulant biocides in the aquatic environment has often been shown to be in the form of a contamination gradient, where greatest concentrations are observed in areas of greatest exposure to boating activity (DOWSON *et al.* 1992;Gomez-Ariza, Morales, and Giraldez 1998). This variation in toxicant spatial distribution can therefore be exploited to study potential ecological responses along contamination gradients. Detailed field-based study of the factors influencing contemporary antifoulant biocide distribution in freshwater systems is therefore required if the scale and extent of such contamination gradients are to be established.

In order to determine the historical variation in TBT contamination, particularly over timescales where regular monitoring has not been performed, analysis of sediment core material has provided such temporal data (Fent, Hunn, and Sturm 1991;Quevauviller, Donard, and Etcheber 1994;Page, Ozbal, and Lanphear 1996;Wade *et al.* 2004;Almeida *et al.* 2004;Scrimshaw *et al.* 2005;Sayer *et al.* 2006). It is the high environmental persistence of TBT once buried within anoxic sediments (Dowson, Bubb, and Lester 1996), that enables the determination of these clear depositionary profiles (Dowson, Bubb, and Lester 1993b).

Analysis of the biological record preserved in accumulated sediments is a palaeolimnological approach which has been successfully used to assess temporal

environmental change in response to stressors on lake ecosystems (Battarbee 1999). As the often rapid shift in alternative equilibria in shallow lake ecology has only rarely been directly observed (Scheffer 1998), palaeolimnological techniques can therefore provide one of the few data sources available for analysis of such phenomena (McGowan *et al.* 2005). Macrophyte community composition can be inferred directly through identification and enumeration of reproductive and vegetative remains, including seeds and leaf tips (Birks 2001). They can also be indirectly inferred through quantification of proxies of macrophyte associated invertebrate taxa, as demonstrated in the work by Brodersen *et al.* (2001) and Odgaard and Rasmussen (2001). Multi-proxy palaeolimnological techniques, where many different biological groups and geochemical proxies, are simultaneously determined from the sediment record have been widely used in the Quarternary sciences (Birks and Birks 1980;Berglund 1986). However such methodologies are becoming increasingly valuable and robust data sources in the determination of factors that have driven environmental change within more recent lake sediments (Brenner *et al.* 1999b;Kenney *et al.* 2002;Eilers *et al.* 2004;Engstrom *et al.* 2006). Multi-proxy analysis is required in shallow lake studies to cross-validate and increase the robustness of palaeolimnological data interpretation, especially where several stressors may have simultaneously influenced the ecology.

1.2 Study Area – The Norfolk Broads

An area where shallow lakes have undergone rapid ecological degradation is the Broads, Norfolk, E. England. This unique wetland area is a popular recreational waterway which consists of approximately 60 man-made lakes, or "broads", created from an extensive peat digging industry that operated up to the 14th century (George 1992). The shallow basins, positioned in the lowland river valley floors, eventually flooded as the excavations were abandoned (Lambert *et al.* 1960). The area has experienced widespread loss of macrophytes and a relatively recent (post 1960s) shift to phytoplankton (Mason and Bryant 1975;Jackson 1978). Due to the variation in navigational access within the Broads, individual shallow lakes have experienced a wide range of exposure to antifoulant biocide contamination (Waldock, Waite, and Thain 1987;Waite *et al.* 1989;Dowson *et al.* 1992;Lambert, Thomas, and Davy 2006). There are also several isolated lake sites, fed by small streams that are up to 3 km from the main waterway.

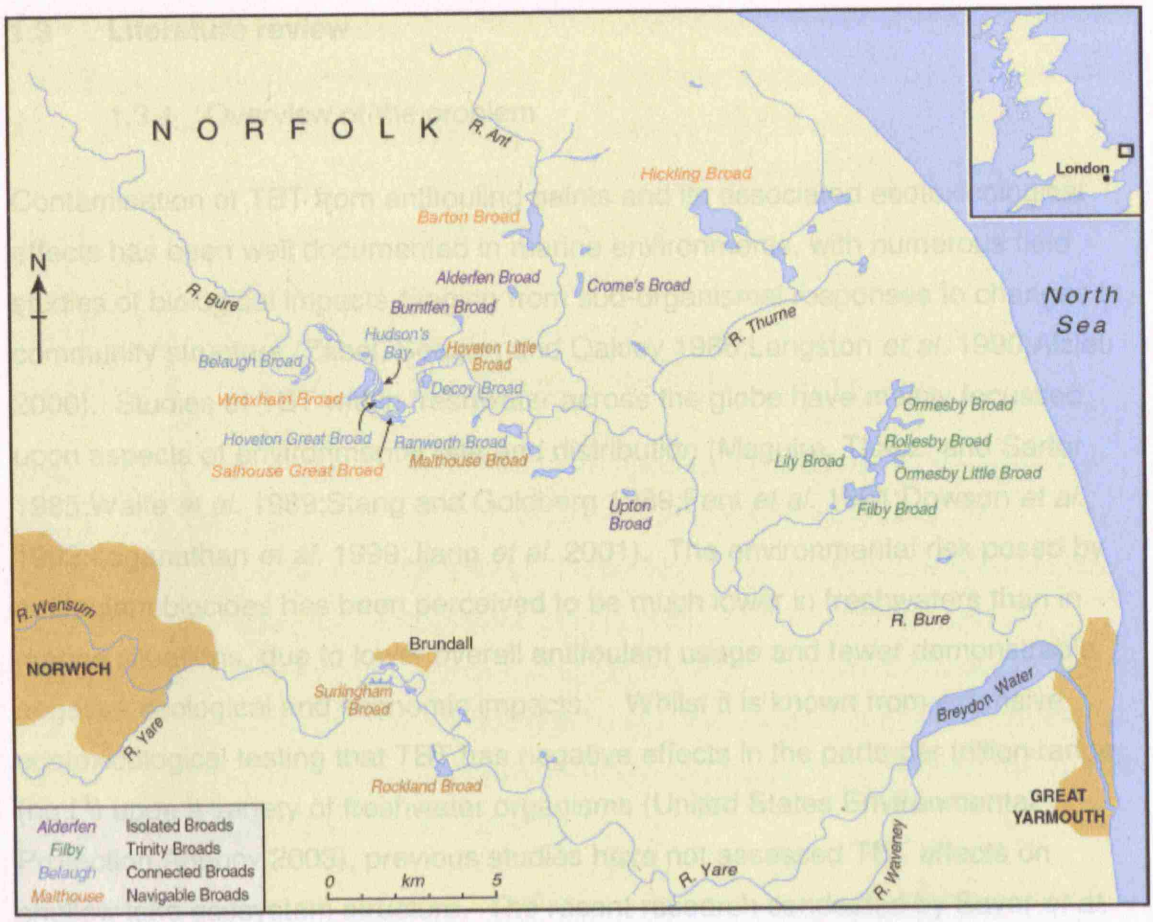

Figure 1.1 Map of the Norfolk Broads and its lowland river system

Research prior to, and following, the 1987 ban on TBT usage on all boats under 25 m revealed widespread water and sediment organotin contamination in the Broads waterways (Boyett 1988;Waite *et al.* 1989;Dowson *et al.* 1992;MAFF 1993;Dowson, Bubb, and Lester 1994). Boatyards, marinas and boat maintenance areas were identified as the major sources of organotins to the Broads aquatic environment, with generally lower concentrations detected in the river channels and broads. More recently a palaeolimnological study of organotin contamination was conducted on a core taken in 2000 from Wroxham Broad, a navigable shallow lake connected to the River Bure (Figure 1.1). TBT concentrations peaked in the core profile at a depth dated as 1973 ± 5 years, with quantifiable amounts occurring up to the surface layers. These findings indicated that TBT was persisting in the Broads aquatic environment. Palaeoecological analysis of biological remains in the Wroxham Broad study also showed distinct changes in the diatom and cladocera community composition, indicative of a switch to phytoplankton dominance, at the same time as TBT was first detected (Sayer *et al.* 2006).

1.3 Literature review

1.3.1 Overview of the problem

Contamination of TBT from antifouling paints and its associated ecotoxicological effects has been well documented in marine environments, with numerous field studies of biological impacts ranging from sub-organismal responses to changes in community structure (Zabel, Seager, and Oakley 1988;Langston *et al.* 1990;Alzieu 2000). Studies of TBT within freshwater across the globe have mainly focussed upon aspects of environmental fate and distribution (Maguire, Tkacz, and Sartor 1985;Waite *et al.* 1989;Stang and Goldberg 1989;Fent *et al.* 1991;Dowson *et al.* 1992;Loganathan *et al.* 1999;Jiang *et al.* 2001). The environmental risk posed by antifoulant biocides has been perceived to be much lower in freshwaters than in marine situations, due to lower overall antifoulant usage and fewer demonstrable negative ecological and economic impacts. Whilst it is known from extensive ecotoxicological testing that TBT has negative effects in the parts per trillion range (ng l^{-1}) upon a variety of freshwater organisms (United States Environmental Protection Agency 2003), previous studies have not assessed TBT effects on shallow lake ecosystem structure. The recent research conducted by Sayer *et al.* (2006) on TBT in the Broads has however questioned previously held assumptions as to the drivers of ecological change in shallow lakes.

The following sections outline the current understanding of the source, fate and ecological impacts of toxic contaminants in freshwater ecosystems.

1.3.2 Types and sources of toxic contaminants in freshwaters

Often substances never designed to be harmful to living organisms have become so, due to their widespread discharge into the environment and particular physio-chemical properties that mean they impair some aspect of biotic functioning. This section gives a brief outline of the types of compound that have the potential to contaminate shallow lakes and elicit some level of ecotoxicological response.

1.3.2.1 Organic pollutants (not used as biocides)

Organic compounds are those that are made up primarily of carbon having various physical structures and additional functional groups. Through developments in

chemical engineering a multitude of entirely novel, entirely man-made, substances have been created over the last century with a diverse range of properties and uses. Many of these xenobiotic compounds have become widespread environmental contaminants. Included in this list are organic compounds that have some natural origins such as polycyclic aromatic hydrocarbons (PAHs) formed as the by-products of incomplete combustion of carbon containing fuels. Increased use of fossil fuels from the industrial revolution onwards has substantially added to the environmental burden of PAH compounds (Lima, Eglinton, and Reddy 2003;Liu *et al.* 2005).

The range of toxic organic contaminants not specifically used as biocides includes; aromatic and aliphatic hydrocarbons, which includes PAHs; polychlorinated biphenyls (PCBs) used as dielectric and lubricating fluids; personal pharmaceutical products; brominated flame retardants; and dioxins. In the European Union legislative measures to limit or cease usage of the most persistent organic pollutants have been introduced through on-going pollution, toxicological and ecotoxicological research that has highlighted the environmental and human risk of exposure to such substances (Regulation (EC) No 850/2004).

Apart from the direct mortality that such toxicants can elicit in high enough doses, sub-lethal effects upon exposed organisms can have impacts throughout aquatic ecosystems. One example where this has been shown to occur is for various endocrine disrupting chemicals (EDCs). These are a diverse range of substances that can influence animal endocrine systems resulting in reproductive, developmental, neurological and immune disfunction (Cooper and Kavlock 2001). These include personal pharmaceutical products and other estrogen-like substances, which have been found to have damaging consequences for fish populations (Parkkonen *et al.* 2000). EDCs act in a variety of ways, mimicking natural hormones, blocking the effects of hormones at receptor sites, or directly stimulating, or inhibiting the endocrine system. The major source of personal pharmaceutical products to the environment has been identified as discharges from wastewater treatment facilities (Gomes, Scrimshaw, and Lester 2003;Rodriguez-Mozaz, Lopez de Alda, and Barcelo 2004).

1.3.2.2 Heavy metals

Living organisms require trace amounts of some heavy metals, including cobalt, copper, manganese, molybdenum, vanadium and zinc, but excessive concentrations can be detrimental to aquatic organisms. Non-essential metals that have no vital or metabolic role, such as mercury, lead and cadmium become toxic to organisms once certain thresholds of exposure and uptake are exceeded (Moriarty 1999). Elevation of these metals concentrations above background levels is often due to human activities. Contamination of heavy metals in shallow water bodies can occur through a variety of ways, among others discharged waste effluents in industrial areas (Kosov and et al 2004), copper leachate from antifoul paints and boat hull cleaning activities (Claisse and Alzieu 1993;An and Kampbell 2003) and mercury from industrial incidents (Bubb, Rudd, and Lester 1991c). Such discharges add to concentrations of metals naturally present in freshwaters derived through surface run-off and groundwater inflows. Atmospheric deposition of metals is another route by which lakes, including those remote from the source of pollution, can become contaminated (Rose and Rippey 2002;Yang *et al.* 2002).

1.3.2.3 Biocides

In contrast to the organic and heavy metal pollutants described above, many synthesised compounds have been specifically designed and used because of their toxic effects upon organisms. Such biocides have come into commonplace usage in a number of agricultural, industrial and domestic applications where unwanted organisms decrease economic or aesthetic value. These include, a vast array of plant protection products, mainly agricultural herbicides and pesticides (Environment Agency 2000) and other biocidal applications on underwater surfaces such as boat hulls, netting and piling; and algicides in industrial water-cooling systems (Bluden and Evans 1990).

Past use of the highly bioaccumulating organochlorine pesticides such as DDT and its metabolites dieldrin and Lindane (used as pesticides in their own right) (Mellanby 1967), has been largely replaced by compounds that are not as environmentally persistent or as prone to bioaccumulation (Lacorte *et al.* 2000). The contemporary herbicide groups such as the phenylureas, triazines and phenoxyacids are used as highly specific agents against economically damaging arable weeds. However, transport from field to watercourses provides a route for exposure of non-target

aquatic organisms. Domestic and municipally used herbicides are also transported to surface waters through storm drain networks in urban areas (Nitschke and Schussler 1998;Gerecke *et al.* 2002).

Prevention of nuisance biological colonisation of underwater surfaces, e.g. on boat hulls and fish farm nets, has been controlled through application of antifoulant paints (AFPs). AFPs are made up of a surface film-forming material; the paint matrix (containing the biocidal ingredients); and a pigment. Continuous leaching of the biocide from the coated surface acts as a toxic barrier to settlement of fouling organisms. This process however also provides a direct route for contamination of toxicants into the aquatic environment. The negative ecological effects of TBT, a potent antifoulant, has been widely studied (Hall and Pinkney 1990;Alzieu 1998a;Hall *et al.* 2000;Maguire 2000;Hoch 2001), especially in marine environments where biofouling is most severe. Organotins have also been specifically studied due to their high potential to cause imposex in molluscs (Alzieu *et al.* 1986;Oehlmann 1996). AFP biocides will be considered in more detail in the next section.

1.3.3 Antifoulant paint biocides

The antifoulant biocide compounds TBT, diuron and Irgarol 1051 are the focus of the present study, as previous work has shown them to be widespread contaminants and in relatively high concentrations within densely boated parts of the Broads study area (Waite *et al.* 1989;Dowson *et al.* 1992;Lambert *et al.* 2006). Mercury, lead, zinc and copper have all been detected in the Broads sediments (Bubb, Rudd, and Lester 1991a;Bubb, Rudd, and Lester 1991b;Bubb *et al.* 1991c;Bennion, Appleby, and Phillips 2001), but along the River Bure, it is the contamination of TBT that has caused the most significant concern as a pollutant (Waldock *et al.* 1987;Sayer *et al.* 2006).

1.3.3.1 Historical overview and biocide evolution

Prevention of colonisation by aquatic organisms or bio-fouling upon underwater structures, particularly boat hulls, has been a challenge for those who work on water for centuries. Increases in the roughness of a vessel's hull creates drag as it moves through water, therefore raising the fuel consumption required to propel the craft. A 10 µm increase in the roughness of an average vessel hull, can cause an increase in

fuel consumption of about 0.3 – 1.0 % (Champ and Seligman 1996). The economic reasoning for use of antifoulant paint thus comes from the increase in fuel efficiency that antifoulant coated vessels, particularly large ocean-going ships, have over non-treated ones. Maintenance costs are also reduced, as there is a lesser requirement for dry-docking and for cleaning of fouled hulls. In marine environments, copper, a metal known to discourage underwater encrustation, was placed along ship hulls in thin sheets as early as the 18th century. By the early twentieth century, more easily applied paints containing a high copper content were developed (Department of the Environment 1974). Copper oxides and other copper compounds have continued to be the primary biocidal agent in most antifoulant paints to this day.

Continuous leaching of biocides from the antifoulant painted surface creates a toxic micro-layer that most aquatic organisms cannot tolerate which therefore effectively prevents colonisation. The initial colonisers on submerged surfaces in marine and freshwaters are bacteria, followed by protozoa and algae with occasional mollusc (*Dreissena polymorpha*) colonisation in freshwaters. Bio-fouling in freshwaters occurs at a slower rate than in marine and estuarine conditions, and is considered less of a problem as fewer freshwater species colonise in this way.

Several species of algae and other microorganisms are tolerant to copper, which has meant that other biocidal compounds have been incorporated into antifoul paints over the years, the so callled "booster" biocides. During the 1960s the discovery that TBT, an already well-known organotin molluscicide, was highly effective when incorporated into antifoul paint, led to it becoming the primary biocide used by manufacturers, often in conjunction with the copper compounds (The Department of the Environment 1986). Data on the types of biocides used historically shows that (in order of greatest amount used); TBT; zinc oxide; the organochlorine insecticide DDT; the carbamate fungicide Thiram; arsenic-based compounds; and mercury compounds; were commonly included in AFP formulations during the early 1970s (Department of the Environment 1974).

Environmental contamination of TBT was first detected in France, where research in the mid 1970s found negative effects of TBT on non-target organisms, particularly farmed oysters around areas that had high densities of small sailing boats (Alzieu 1998a). Symptoms of TBT exposure among marine molluscs include shell thickening and deformities; imposex; and reduced survival of larvae (Alzieu *et al.*

1986). Such findings led to a ban in France of TBT usage on small boats <25 m in 1982. In the UK use of TBT antifoulant paint on small boats and aquaculture was banned in 1987 (under section 3(3) of the Control of Pesticides Regulations, 1986). Similar legislation followed in the USA in 1988, and with many other European countries around 1990. Several studies have shown that the water concentration of TBT significantly reduced after these bans (MAFF 1993;Dowson *et al.* 1994).

After the 1987 ban on TBT usage, replacement booster biocides were subsequently used and developed specifically for use in antifoul coatings. The UK Health & Safety Executive (HSE) list of approved biocides included at that time the organic herbicides diuron and Irgarol 1051, the fungicides dichlofluanid and chlorothalonil, and several other organic and organo-zinc compounds (Thomas 1998). Of these biocides the most widely used were diuron and Irgarol 1051 (Environment Agency 1998). In the UK, the Advisory Committee on Pesticides (ACP) conducted a review of the acceptability of the use of "booster" biocides in antifoul paint formulations in 2000. As a result, the amateur use of Irgarol and diuron was revoked for use on all boats <25 m in length, with the ban effective from 2002 and 2003 respectively. This was due to concerns over the tighter restrictions on TBT use as an antifoulant would lead to the increased environmental loading of these organic biocides, and also the negative human health consequences for amateur user's when handling the products (HSE 2001).

The greatest environmental contamination of antifoul biocides has been found in areas of dense boat moorings and where maintenance activities occur (Waite *et al.* 1989;Dowson *et al.* 1992;Hall *et al.* 1999;Biselli *et al.* 2000;Basheer, Tan, and Lee 2002;Gardinali *et al.* 2002). Temporal trends in biocide concentration over the course of a boating season have also been observed; with highest water concentrations recorded when freshly painted boats are simultaneously returned to the water (Bowman, Readman, and Zhou 2003). The available literature on organotins and the subsequently used organic booster biocides is dominated by environmental contamination and ecotoxicity in estuarine and seawaters, especially around areas of high boating/shipping activity (Hoch 2001). Reported freshwater contamination of antifoulant biocides is less widespread in the literature than for marine environments, but the risks posed to navigable freshwater ecosystems are highly relevant for nature conservation, sustainable water resource management and environmental regulatory compliance.

1.3.3.2 Alternative sources of AFP biocides to the environment

Organotin compounds are a wholly synthetic family of chemicals characterised by the presence of one or more tin-carbon bonds. Their general form follows the formula R_nSnX_{4-n}, where R is an aryl or alkyl group, X is an anionic species, eg. chloride, oxide, hydroxide or other functional group, and n is 1 to 4. Table 1.1 lists some of the common organotin applications.

Table 1.1 Application and usage of organotin compounds

Application	Function	Principal compounds used
Tributyltins – Bu_3Sn-X		
Wood preservation	Fungicide	bis(tributyltin) oxide
		tributyltin naphthenate
		tributyltin sulphate
		tributyltin flouride
		tributyltin phosphate
Materials protection	Fungicide	bis(tributyltin) oxide
(stone, leather, paper etc)	Algicide	tributyltin benzoate
Disinfection	Bacteriostat	tributyltin benzoate
Schistosomiasis control	Molluscicide	bis(tributyltin) oxide
Antifouling paints	Biocide	bis(tributyltin) oxide
		tributyltin flouride
		tributyltin chloride
		tributyltin acetate
		tributyltin acrylate polymers
Dibutyltins – Bu_2Sn-X		
Plastics stabilisers	Heat & light effects	dibutyltin di-isooctylthioglycolate
		dibutyltin maleate
Polyurethane foams	Catalyst	dibutyltin diacetate
Poultry management	Dewormer	dibutyltin dilaurate
Monobutyltins – BuSn-X		
Plastics stabilisers	Heat & light effects	monobutyltin tri-isooctylthioglycolate
Glass industry	Tin (IV) precursor	monobutyltin trichloride
Triphenyltins – Ph3Sn-X		
Agricultural chemicals	Fungicide	triphenyltin acetate
	Insecticide	triphenyltin hydroxide
Antifouling paints		triphenyltin flouride
		triphenyltin chloride
		triphenyltin acetate
Moth proofing	Insecticide	triphenyltin chloride
	Antifeedant	triphenyltin hydroxide

References: (Bokranz and Plum 1975;Blunden and et 1984;Zabel *et al.* 1988;Bluden and Evans 1990).

AFPs are just one of the many commercial applications of organotin compounds. The original use of organotins, particularly dibutyltin (DBT), as heat and light stabilizers for PVC and other plastics, continues to be the greatest (by mass) industrial usage (Bluden and Evans 1990). Use in AFPs has provided a direct route for TBT to the freshwater aquatic environment (Maguire *et al.* 1982;Dowson *et al.* 1992;Fent and Hunn 1995), but the widespread and varied uses of organotins has resulted in other, albeit lesser, but nonetheless relevant, contamination sources. TBT, DBT and monobutyltin (MBT) have been detected in municipal wastewater discharges and sewage sludge (Fent and Müller 1991;Diez, Abalos, and Bayona 2002); landfill leachate; and in discharges or accidental spillages from commercial timber treatment plants (Schebek, Andreae, and Tobschall 1991;Bailey, Owen, and Davies 1997).

Diuron is a phenylurea herbicide frequently used for the total removal of plant growth from hard surfaces, such as pavements, roads, railway lines etc (Lewis and Gardiner 1996). Its mode of action is to block electron transport at photosystem II (PSII) which acts to inhibit photosynthesis. Its broad-spectrum efficacy means it kills broad-leaved plants, grasses, moss and algae. It also has applications for weed control in soft fruit orchards, forestry and between ornamental trees and shrubs where it can be used as a residual herbicide (Whitehead 2001). Diuron contamination of surface waters has been found to occur through run-off from urban areas (Blanchoud et al 2004).

Irgarol 1051 use is solely restricted to use in antifoul paint formulations. It is a triazine herbicide, a herbicide family that also includes the common agricultural herbicides atrazine and simazine. Irgarol is also a PSII inhibitor and is used in AFPs primarily for its algicidal properties.

1.3.4 AFP biocide environmental fate

Once delivered into a shallow water body, toxic contaminants will generally be subject to physical partitioning between water, sediment, biotic and/or atmospheric compartments. Some of these compartments may take up relatively little of the substance, through processes related to the particular physico-chemical properties of the substance involved, and the form in which it was delivered to the environment. Highly soluble chemicals will generally remain in solution, but hydrophobic

compounds like TBT are more likely to become bound with solid phases (Dowson, Bubb, and Lester 1993a). For this reason, sediments can become highly contaminated even if the original discharge was delivered in solution. The wider spatial distribution of contaminants is also dependent upon the movement of the physical compartment a substance finds itself in, such as through water currents or sediment transport.

The environmental persistence of AFP biocides is related to their degradation, by chemical, physical and/or biological processes (Callow and Willingham 1996). The type of phase a substance is associated with determines its breakdown pathway, which leads to observed variation in contaminant half-life between different phases (Maguire and Tkacz 1985). Additional variation in degradation rates can occur through the effects of seasonality, heterogeneity in the physical environment and/or fluctuations of pH, temperature and oxygen (Lester, Bubb, and Lester 1996;Okamura *et al.* 1999). These factors combine to determine a contaminant's half-life in a particular phase, which is defined as the time required for the quantity to fall to half of its initial amount. Important processes that break down or transform biocides in freshwaters include photolysis and biological metabolism (Comber *et al.* 2001). As such, AFP biocide half-life is generally shorter in water than when associated with sediments (Waite *et al.* 1989). Once buried in deeper sediments light and oxygen are not as readily available as degrading agents, and also there can be can be a reduced abundance of common microbial groups that account for much toxicant breakdown (White, Tobin, and Cooney 1999). Partitioning and degradation combine to determine the environmental availability of AFP biocides to organisms, ie. the bioavailability, and hence the potential for bioaccumulation and/or possible ecotoxicological effects.

1.3.4.1 Antifoulant biocide water - solid partitioning

Once leached from an AFP, dissolved TBT experiences change in charge and attachment of different functional groups, depending on the chemistry of the receiving water body. The predominant form in freshwaters is the neutral TBT-OH species, but possibly as the cationic TBT^+, depending on the pH of the receiving water (Arnold *et al.* 1997).

Hydrolysis of the TBT-X compound has been found to behave similarly to a weak acid, whereby at pH < pK_a (hydrolysis) the dissociation reaction can be described as:-

$$\underset{pH > pK_a}{TBTOH} \leftrightarrows \underset{pH < pK_a}{TBT^+ + OH^-}$$

A stability constant of log$K$ = 6.51 reported by Shoukry (1993) cited in Fent and Looser (1995) would mean that at pH < pK_a = 6.51, the dominant TBT species would be the cation TBT^+. Whereas at pH > 6.51 the neutral TBTOH would be most common. A pK_a value of 6.25 for TBT reported by (Arnold *et al.* 1997) is in close agreement with the previously reported pK_a value. In addition to pH, the concentration and type of anions, and temperature also influence TBT speciation. In their work on TBT speciation Arnold *et al* (1997) found all the monovalent anions studied formed much weaker complexes with TBT than the relatively stable hydroxide.

Speciation is important as it influences adsorption processes and thus how TBT partitions in the aquatic environment. Hydrophobic partitioning of TBT occurs to natural organic matter (Bluden and Evans 1990) and combined with sorption to mineral fractions such as clays (Weidenhaupt *et al.* 1997), gives the total sorption from the aqueous to particulate phase. A recent review of TBT contamination by Hoch (2001) reported that environmental TBT concentrations in the aqueous phase are generally an order of magnitude lower in than in the sediment. Sediments therefore act as sinks for organotin compounds in aquatic environments. Laboratory-based experimental work has shown that equilibrium partitioning between water and sediment, measured as Kd (partition coefficient), shows that TBT+ would be expected to preferably adsorb to particulate matter, but the reverse process is also possible (Watanabe, Sakai, and Takatsuki 1995). Adsorption can be to suspended or bed sediments (organic and inorganic material), humic substances and biota. From octanol – water partition coefficient values in the literature, logK_{ow} for TBT range from 3.7 – 4.4 (see Table 9.5, Appendix 9.1) and thus TBT is expected to sorb to organic matter in freshwaters. This prediction has been supported experimentally with organic rich sediment collected from the River Yare in Norfolk removing up to 100% of TBT delivered in solution (Dowson *et al.* 1993a).

This study also found that the proportion of suspended particulate matter increased the relative amount of TBT removed from solution.

The organic booster biocides Irgarol and diuron are less hydrophobic than TBT, with reported log K_{ow} values ranging from 2.8 – 4.1 for Irgarol and a single value of 2.8 for diuron (see Appendix 9.1). Studies have indicated that Irgarol, and particularly diuron, are more likely to be present in the aqueous phase in natural waters, than associated with suspended or bed sediments (Thomas *et al.* 2002;Voulvoulis *et al.* 2002;Comber *et al.* 2002). However, in areas with significant AFP usage, contamination of Irgarol and diuron in sediments has been found (Tóth *et al.* 1996;Thomas, Blake, and Waldock 2000;Voulvoulis, Scrimshaw, and Lester 2000;Albanis *et al.* 2002;Bowman *et al.* 2003). Environmental properties such as suspended sediment concentration and sediment type have shown to influence the relative amount of partitioning to the sediment phase. For example, increased sorption of biocides to sediments containing greater organic matter contents and greater total biocide sorption at increased suspended sediment concentrations have been observed (Voulvoulis *et al.* 2002;Comber *et al.* 2002).

1.3.4.2 Biocide environmental persistence

It is generally accepted that degradation of TBT follows stepwise de-butylation ultimately to elemental tin, with the intermediate formation of dibutyltin (DBT) and mono-butyltin (MBT), both of which have been found in the aquatic environment (Maguire and Tkacz 1985;Cleary and Stebbing 1987a;Loganathan *et al.* 1999). Environmental breakdown of TBT occurs through two main processes, photolysis and biological degradation (Dowson *et al.* 1996;Hoch 2001). Reported half-lives of TBT in water show wide variation, ranging from 3.5 days (Hinga, Adelman, and Pilson 1987) to 360 days (Dowson *et al.* 1996). A study of the direct photolysis of TBT found that degradation was slow, with a half-life of 90 days (Maguire, Carey, and Hale 1983). However, reported half-lives are highly dependent upon the conditions within which the degradation was studied (Waite *et al.* 1989), and some of the very short half-lives reported for water could be more accurately described as measures of partitioning rates, with loss of TBT to the sediment or other phases rather than biological or physical decomposition of the TBT compound itself.

TBT therefore appears to be more persistent in sediments than in overlying water (Maguire 1992), especially under anoxic conditions, with the longest freshwater sediment TBT half-life reported to be in the order of decades (Dowson *et al.* 1996). Dowson *et al* (1993) concluded that biological processes were the most important mechanism for the decomposition of TBT in aquatic ecosystems. However, in a laboratory study by (Kawai *et al.* 1998), certain microbial strains that actively decomposed TBT were themselves inhibited at higher TBT test concentrations, which may mean that increased persistence of TBT could occur in the most heavily contaminated areas.

For AFP biocides the primary fate processes influencing environmental persistence are water-sediment partitioning, degradation and transport processes (Thomas *et al.* 2002). For the organic biocides, the latter may be of particular importance, due to the their greater association with the water phase, especially in tidally influenced riverine systems where there are daily large scale horizontal water movements. The influence of biocide transportation has been highlighted by continuously greater biocide concentrations observed within enclosed marinas (with lower water exchange rates) compared to similar sized marinas open to tidal action (Biselli *et al.* 2000;Sargent, Bowman, and Zhou 2000). The resultant distribution away from source areas of the more soluble organic AFP biocides, may therefore be greater than for deposited, sediment-associated TBT.

In a review by Hall *et al* (1999) the half-life of Irgarol reported in freshwater ranged from 36 days for photolysis and 96 days under aerobic metabolism. It is generally regarded as having a slow degradation rate in both sea and freshwaters (Konstantinou and Albanis 2004). Studies have identified Irgarol degradation products in the environment (Martinez, Ferrer, and Barcelo 2000;Martinez and Barcelo 2001;Lambert *et al.* 2006), with the primary product (2-methylthio-4-*tert*-butylamino-6-amino-s-triazine, known as GS26575 or M1) formed through biological, chemical, and photo- degradation (Liu *et al.* 1997;Okamura *et al.* 1999;Okamura 2002). Hall et al (1999) concluded that the principal degradation processes affecting Irgarol in the environment would be microbial metabolism under aerobic conditions and photolysis.

In a review of diuron persistence, no studies were found that reported a half-life representing its degradation in freshwaters (HSE 2001). The overall description of

diuron was that it is hydrolytically stable over the range of pH likely to be found in UK waters and that significant abiotic degradation in the aquatic environment was unlikely. However, numerous laboratory studies have demonstrated microbial biodegradation of diuron (Giacomazzi and Cochet 2004). Diuron degradation products have been detected in marine surface waters and bottom sediments (Thomas *et al.* 2002). Increased persistence of AFP biocides was found to occur in sediments where AFP flakes were present, as continual leaching of fresh biocide from the paint matrix maintained environmental concentrations (Thomas *et al.* 2002).

1.3.5 Ecological consequences of toxic contamination

The sources of AFP biocides and their environmental fate have been discussed. The next section gives a brief summary of the key ecotoxicological effects experienced by aquatic biota from exposure to toxicants with particular reference to TBT. This particular AFP biocide is considered in the present study as it is the ecological impacts of TBT observable in the paleaoecological record that is of primary interest, especially in Chapter 6. The toxicity of the more recent (post-phytoplankton dominance) organic booster biocides are therefore not considered in the present study.

1.3.5.1 Organism exposure to toxicants

For a chemical stressor to illicit an ecotoxicological response from biota, a contaminant released into the aquatic environment must enter an organism and reach an active site within the tissues where a response can occur. At the cellular level, TBT inhibits Na+ and K+ ATPases that are ionophores controlling exchange of Cl-, Br-, Fl- and other ions across cell membranes (Selwyn 1976). Toxicants may be taken up through respiratory surfaces (e.g. gill, lung); general body surface (e.g. skin, cell walls); or oral ingestion (e.g. food, water). The fastest route is often via the respiratory surfaces because of their efficient absorbtive characteristics and the high surface area of these tissues (Connell *et al.* 1999). As aquatic organisms by definition live partially or totally submerged in water for all or part of their life cycle, dissolved contaminants pose the greatest risk to such organisms, as uptake from the water medium can occur directly into the tissues where the toxic agent can begin to act.

After entering an organism, toxicants can be eliminated in several ways. This may be achieved through excretion or transformation into different chemical forms through various metabolic processes. Many microbial and fungal species are able to transform toxicants, for example TBT is biodegraded and transformed by microalgae (Tsang *et al.* 1999) and similar biodegradative processes have been observed in natural river sediments (Landmeyer 2004). Therefore if a toxicant can be effectively transformed and eliminated from the exposed organism, the potential toxicological effects are reduced. However, it has been widely shown that TBT accumulates in directly exposed organisms (Huang *et al.* 1993;Tas and Opperhuizen 1995;Fent and Looser 1995;Maguire 2000;Bartlett *et al.* 2004) and that it is also transferred through trophic levels in the food web (Traas *et al.* 1996;Veltman *et al.* 2006). Alarmingly, evidence of TBT accumulation in top mammalian predators, such as otters and river dolphins, has been reported (Kannan, Senthilkumar, and Sinha 1997;Kannan *et al.* 1999). Biomagnification of lipophilic substances, such as TBT, occurs as lipids are relatively metabolically inactive compared to other body tissues and therefore contaminants can remain undegraded within organisms (Connell *et al.* 1999). For toxic substances that have long biological half-lives like TBT, this can pose serious environmental risks, as an organism's long-term exposure is increased and bioaccumulation can occur even when ambient environmental concentrations are relatively low (Laughlin 1987).

TBT bound to sediment particles poses a threat mainly to organisms that live in or feed upon the sedimentary material (Bartlett *et al.* 2005). The process of equilibrium partitioning between sediments and interstitial pore waters means that TBT pore water concentration can potentially reach much higher levels than in overlying surface waters (Ansari, Singh, and Tobschall 1998). This increases the risk to such benthic dwelling or benthic feeding organisms further as the soluble TBT is more bioavailable. A route by which non-sediment dwelling organisms can become contaminanted by substances primarily associated with the sediment, is by feeding upon benthic organisms. Food web interactions can therefore lead to more widespread bioaccumulation in organisms not directly exposed to the sediment-associated contaminant.

1.3.5.2 Testing for ecotoxicological responses

Determination of the toxicity of environmental pollutants has traditionally been carried out in standardised laboratory tests on cultured or field-collected organisms. This methodology allows most external variables to be controlled giving high quality data on the specific toxicological effects, or end-points being measured. This type of toxicity test data provides the basis upon which Environmental Risk Assessments are made for TBT and other toxicants (van Wezel and van Vlaardingen 2004a;Leung *et al.* 2005b). Sub-lethal responses in individual test organisms include a variety of potential modifications at the molecular, biomolecular, physiological and behavioural levels. Such sub-lethal measures however tend not to have the informative power to translate up from the individual organism response to population and higher organisational levels (Connell *et al.* 1999) which are further considered in this study. Such measures are, however, very sensitive and can be used as early signs of stress to a toxicant.

The vast range and inumerable combinations of substances that it is possible to test by standard toxicological methods means often only the simplest single species test data is available for any given pollutant (EU 1997). The species used in such tests may not be entirely representative of those found in specific natural waters and are unlikely to represent the most sensitive ones (Cairns 1986). However, tests that look for changes in the Darwinian fitness traits of growth, reproduction and survival provide a much better conversion from individual responses to effects at the higher organisational levels of population, community and ecosystem (Selck *et al.* 2002). Additionally, variation in toxicant sensitivity between life stages of organisms can have marked effects on populations, as modifications to population age structure influences longer term reproduction cycles. For example, juvenile stages have been shown to be more sensitive to a wide range of toxicants than mature individuals for a range of organism groups including cladocera (Hanazato 2001), copepods (Wendt-Rasch *et al.* 2003), macroinvertebrates and fish (Hutchinson, Solbe, and Kloepper-Sams 1998)

As the level of biological organisation increases from population, community to ecosystem, the level of complexity and variability also increases. This means only large-scale ecological changes are visible against what is usually naturally high background variability. Detecting such ecological changes also relies on there being

little compensation or homeostatic mechanisms within the level of organisation under observation, such as rapid reproduction or migration of new individuals acting to increase population abundances. The number, complexity and variety of ecological interactions increases within successively higher levels of biological organisation, ie. population, community, through to the ecosystem level. This makes it difficult to translate how direct toxic effects upon an affected population will have knock-on impacts upon other organism populations present in that community or ecosystem (Luoma *et al.* 2001). Such limitations however can to some extent be lessened through a priori knowledge of the system under observation. Section 1.3.6 details some of the ecological relationships common in shallow lakes.

Micro- and mesocosm studies used as part of higher-tier risk assessments aim to act as a bridge in ecotoxicological understanding between laboratory based, single species toxicity tests and field effects of toxicants (Traas *et al.* 2004). Organisms exposed to toxicants in their natural surroundings may have different sensitivities compared to laboratory test conditions because of factors such as reproductive traits that are density dependent and stress induced by food shortage (Wendt-Rasch *et al.* 2003). Single species laboratory tests do not incorporate alterations to ecosystems resulting from indirect ecological effects of toxicant impacts. This aspect of toxicant impact on wider ecosystems structure and functioning is discussed in section 1.3.5.4.

1.3.5.3 Known ecotoxicological effects of TBT

TBT is acutely toxic to many aquatic organism groups, particularly mollusca. Numerous references to TBT toxicity upon marine and freshwater organisms exist in the literature and have been previously reviewed (Zabel *et al.* 1988;Oehlmann 1996;Hall *et al.* 2000;Maguire 2000;Selck *et al.* 2002;United States Environmental Protection Agency 2003). The most comprehensive collation of this data was accessed via the US EPA online database ECOTOX (http://www.epa.gov/ecotox/ecotox_home.htm accessed 27/08/2004). A full table of all freshwater tests from the ECOTOX database that show negative effects of TBT (at water concentrations reported from the Broads) is included in Appendix 9.6.

Various reviews have cited the impacts of marine TBT contamination as a clear example of pollution induced change within benthic ecosystems and a causative

agent of imposex in molluscs through its endocrine disrupting properties (Alzieu 1998a;Maguire 2000;Harding and Davies 2000). In this section a review is made of the studies that have shown direct and indirect population and community level responses to ambient environmental TBT concentrations.

TBT has been shown to influence the structure and functioning of various biological communities through mesocosm studies in the marine environment. However, such a well established body of research detailing the population and community level effects of TBT exposure in the freshwater environment is unfortunately lacking. Some salient points regarding the ecological interactions between organisms following TBT exposure can however be taken from these analagous aquatic ecosystems.

Studies have shown that structural changes to natural phytoplankton assemblages can occur following TBT exposure at ambient concentrations in coastal waters (Petersen and Gustavson 2000). The degree of assemblage change in long-term exposed communities has been shown to display population induced community tolerance (PICT) responses in both phytoplankton (Blanck and Dahl 1996;Petersen and Gustavson 1998) and periphytic diatoms (Molander *et al.* 1992). Such changes are indicative of a community wide response to damaging exposure levels, through the reduced abundance of the most TBT sensitive species populations. Significant decreases in total abundance, decreased diversity and assemblage change have also been observed within TBT exposed marine benthic macro- and meiofaunal communities (Dahllof *et al.* 2001;Schratzberger *et al.* 2002). Changes to zooplankton community functional attributes such as reduced grazing on phytoplankton have been observed from relatively low TBT concentrations (Molander *et al.* 1992;Jak *et al.* 1998a).

These marine studies highlight the importance of the indirect effects, not just direct lethality, that TBT toxicity can have on otherwise insensitive biological groups. The marine studies demonstrate how TBT can radically modify the overall structure and functioning of the exposed ecosystem.

1.3.5.4 Direct and indirect ecological effects

In addition to the marine findings in the previous section, freshwater community responses to exposure of toxicants other than TBT have been reported from mesocosm or exclosure studies. Some general ecological principles regarding response to toxic contamination will be outlined in this section.

Direct toxicity to sensitive species, or sensitive individuals within a population, often has the overall effect of reduction, or possibly elimination of that population. The spatial extent of toxic effects depends upon the bioavailability of a toxicant, which is determined by the properties of the toxicant, the quantity that is present in a given area and also the ecosytem properties, which control rates of partitioning, transportation and degradation. However the duration of toxicant impacts depend not only upon bioavailability and sufficient concentration, but also species specific recovery factors. Species recovery depends upon influx of new recruits (migration) and the species reproduction rate. In shallow lakes organism groups such as cladocerans have a fast regenerative capacity, with a reproductive cycle in the range of days to weeks (Gyllstrom and Hansson 2004), compared to slower breeders like fish that may only spawn once a year. The regenerative capacity of impacted species, or species groups, therefore influences the duration of observable effects in the environment, whether they were caused directly (such as mortality from lethal exposure levels) or indirectly (through modification of trophic relationships). The degree of ecological connectivity of an impacted site is also important for how fast recovery occurs, as those isolated from source pools of new recruits, will have lower recovery potential.

Indirect effects occur through modification of the abiotic environment and/or alterations to ecological relationships. Toxicant exposure has been shown to modify interspecific and trophic relationships between species in mesocosm studies (Friberg-Jensen *et al.* 2003;Traas *et al.* 2004). Change in one species population, e.g. one sensitive to a toxicant, can have concomitant effects in another species populations if there is a direct trophic link between them. For example *Daphnia* are generally more sensitive to many toxicants compared to fish (Hutchinson *et al.* 1998), therefore after a severe pollution event, reductions in the cladocera prey will influence the food available to zooplanktivorous fish. Furthermore, phytoplankton

consumed by *Daphnia* will be released from grazing pressure leading to an increase in phytoplankton population size (Carpenter and Kitchell 1994).

Variation in sensitivities to toxicant stress within functional groups is also common. For example Jak *et al.* (1998c) tested the response of a natural zooplanktonic grazer community to exposures of 3,4-dichloroaniline, a common herbicide breakdown product. They found that tolerant copepod species benefited from population reductions of the more sensitive cladoceran species, as they were released from interspecific competition for the same algal food resource. This example of functional redundancy within an ecosystem, where one group of organisms (copepods) replaces the functional role (algal grazing) of a group that has been removed/reduced (cladocerans), is often seen as a way in which ecosystem resilience is maintained. However due to the wider food web interactions affecting all species and differing ecological strategies of aquatic taxa, is is highly unlikely that all functions and trophic relationships of organisms will be maintained in the face of long term toxicant exposure.

The wide range of indirect effects and homeostatic ecological changes that can occur within shallow lakes, mean that the negative impacts of toxicants may become difficult to identify. The study of shallow lake ecology, mainly from attempts to restore pre-disturbance conditions, has shown that such biotic interactions and trophic cascades play very influential roles in determining overall ecosystem structure (Carpenter, Kitchell, and Hodgson 1985;Brönmark 1994;Blindow *et al.* 2000;Perrow, Moss, and Stansfield 2004). Also the complexity of biological interactions means that if only one or two biotic groups are considered in a study of a toxicant's environmental impact, the true results are more likely to be masked or open to misinterpretation.

A stress is defined in this study as an environmental factor, which can potentially cause a significant change in an organism, population, community or indeed whole ecological system. Such stressors may act simultaneously or sequentially, and can be termed "complex stressors". These stressors may interact in ways that make it difficult to isolate any single factors contribution to an observed biological response, especially at the ecosystem scale (Dorward-King *et al.* 2001). This complexity in unravelling cause and effect of impacts at the ecosystem scale requires

development of research methodologies that can demonstrate that such ecological changes have occurred.

1.3.5.5 Effects of TBT in the Broads

This section gives a brief summary of ecotoxicological effects of TBT exposure at concentration levels that existed within the Broads during the period of active usage.

Within the Broads, a few previous studies have indicated that TBT may have had negative ecological effects. During 1989 the maximum water TBT concentration measured in the River Bure system was 1620 ng l^{-1} from a boatyard in the village of Wroxham and in Wroxham Broad itself a maximum of 898 ng l^{-1} was detected during the same year (MAFF 1993). A mesocosm experiment involving TBT dosing (max. conc. 600 ng l^{-1}) of established zooplankton and mollusc communities in Hoveton Great Broad showed significant reductions in the overall grazing rates of zooplankton and reduction in mollusc populations after TBT additions (Kerrison 1988;Kerrison 1989). Work by Jackson (1999) has shown that numbers of sensitive invertebrate species, particularly molluscs, were depressed in navigable areas of the Norfolk Broads at the time of peak TBT usage. These Broads-based studies are supported by the wider freshwater ecotoxicological literature. At the maximum recorded dissolved TBT concentrations observed along the River Bure, sensitive species of mollusc; crustaceans, including zooplankton; phytoplankton; Dipteran midge larvae; and fish would have experienced lethal toxicological effects capable of negatively affecting their population levels. Appendix 9.6 contains a list of the relevant freshwater ecotoxicological test responses and exposure levels from which these former examples are drawn.

1.3.6 Shallow lake functioning and potential impact of toxicants

Given the potential range of ecotoxicological responses within aquatic ecosytems, particular focus upon the key structural and functional aspects of shallow lake ecology is required prior to study of toxicant impacts in such habitats.

Shallow lakes in temperate regions are polymictic, having a mixed water column throughout the year and are more susceptible to the inflow of nutrients than deep lakes. They occupy a position in generally nutrient and organic matter rich lowland

areas; their low volume generates a higher loading per unit area; and soluble nutrients are well mixed throughout the water column (Wetzel 2001). Pristine shallow lakes are often characterised by having light penetration through the greater part of the water column and low phytoplankton productivity. The influence of submerged macrophytes upon ecological functioning is much greater in shallow compared to deep lakes, as most of their basin area can be potentially, and often is, colonised by submerged macrophyte growth (Scheffer 1998). Naturally occurring biotic and abiotic sources of turbidity do occur (Coops *et al.* 1999), but the water depth is often sufficiently shallow to allow macrophyte growth to persist. Such natural turbidity and the subtle variation in water depths in shallow lake basins, lead to the observed heterogenous distribution of macrophytes (Körner 2001) and the diversity of species that are able to co-exist (Tremolieres 2004). The physical habitat provided by macrophyte growth in shallow lakes supports a diverse community of periphytic and filamentous algae. In turn this is utilised as a resource by invertebrate grazers (molluscs, microcrustacean cladocerans and aquatic insects in various life stages) themselves consumed by other predatory invertebrates, fish and birds, with the complete web of trophic interactions being complex and interlinked (Jones and Waldron 2003).

Phytoplankton productivity is generally low within pristine, low nutrient condition shallow lakes, as competition for the rapidly and efficiently cycled nutrients generally favours macrophytes. The sediment acts as a nutrient store in shallow lakes, which rooted plants can exploit (Moeller, Burkholder, and Wetzel 1988) and luxury uptake of excess soluble nutrients is also a common strategy of the higher plants (Lee and McNaughton 2004). Furthermore, zooplankton grazers actively feed upon phytoplankton and periphytic algae, often exerting strong control over total algal abundance (Jeppesen *et al.* 1999). These kinds of trophic cascades are particularly strong in shallow lakes. Both top-down (predator/herbivore influence on lower trophic levels) and bottom-up (nutrient/food/prey influence on higher trophic levels) processes act, to varying degrees, in shaping the overall biological structure within shallow lakes (Jeppesen *et al.* 2003;Stephen *et al.* 2004a).

Macrophyte growth acts to stabilise the potentially flocculent and unconsolidated surface sediments of shallow lakes in a number of ways. For example, wave-induced resuspension of this material is suppressed through increased flow resistance from plant biomass within the water column and the presence of root

networks within the sediment itself (James, Barko, and Butler 2004;Horppila and Nurminen 2005). The stabilisation effect of macrophytes helps to maintain a favourable light climate for their continued growth. Thus a plant dominated ecosystem state persists through time via several positive ecological feedbacks (Moss *et al.* 1996a). In this way shallow lakes can be said to have a natural buffering capacity, or resilience, against both internal fluctuations from within their own ecosystem processes and from minor external perturbations.

The likelihood of a switch between states is greater if there is already a reduced resilience within the ecosystem, as it will be less able to recover from natural or human-induced fluctuations (Scheffer *et al.* 2001). Progressive cultural eutrophication has the effect of increasing the propensity of a shallow lake to move into the turbid state with eventual loss of macrophytes.

However, alternative equilibria theory suggests that a perturbation is required prior to the wholescale loss of macrophytes, as opposed to increased nutrients causing this alone (Scheffer *et al.* 1993;Scheffer 1998). This process is summarised by the marble-and-cup diagram in Figure 1.2. As ambient nutrient concentrations increase and the lake condition heads towards point F1, the resilience of the macrophyte dominated equilibria (represented by the valley on the left of the diagram) becomes weaker. At this point, often only a minor perturbation is required to switch the shallow lake ecosystem to a turbid, phytoplankton dominated state represented by the line beginning at point F2 and move the ball in the right hand valley (Scheffer *et al.* 2001).

Perturbations can be grouped as those that directly remove macrophytes, usually in the form of large scale stochastic events; or alternatively more subtle ecological factors that indirectly promote algal dominance (Moss *et al.* 1996a;Jones and Sayer 2003). Specific examples of where stochastic perturbations have caused a direct loss of macrophytes and a subsequent switch to phytoplankton dominance include: man-made water-level changes (Bengtsson and Hellström 1992;Blindow *et al.* 1993); introduction of grass carp and addition of herbicides (Richard, Small, and Osborne 1984); introduction of herbivorous crayfish (Rodríguez, Bécares, and Fernández-Aláez 2003); and violent storms (Mitchell 1989;Schelske and Brezonik 1992).

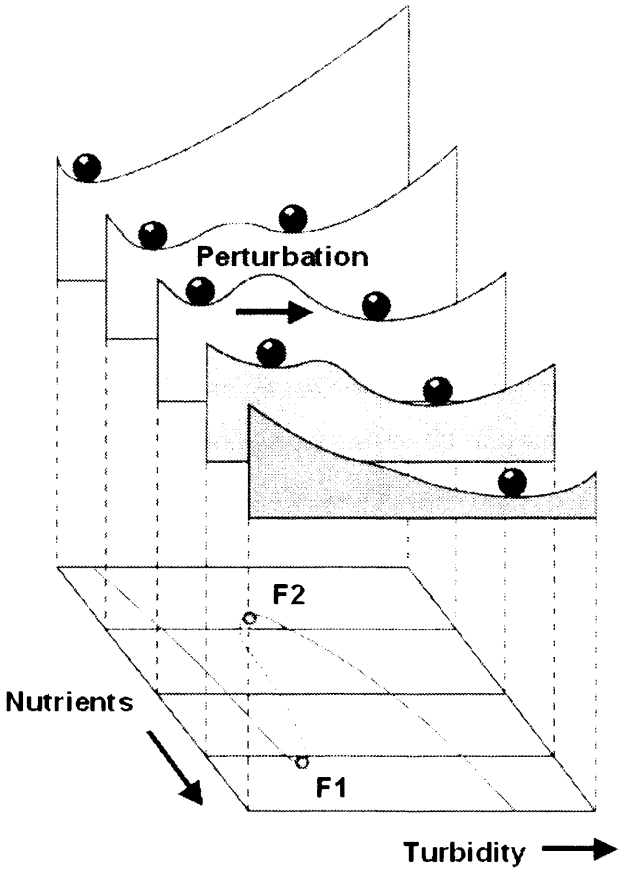

Figure 1.2 Marble-and-cup diagram of how external conditions affect the alternative equilibria in shallow lakes
(Modified from (Scheffer *et al.* 2001))

The rapid and widespread loss of submerged macrophytes from the Norfolk Broads has often been attributed to the increase in phosphate load entering the waterways at that time (Phillips, Eminson, and Moss 1978). However, it is unlikely that all shallow lakes that have switched to phytoplankton dominance will have experienced very high nutrient levels (Jones and Sayer 2003). As the loss of macrophytes can apparently occur over a wide range of total phosphorus concentrations (Jeppesen *et al.* 1991), a perturbation must therefore also disrupt the ecosystem to bring about the observed catastrophic change, if the alternative stable state theory is to be satisfied (Figure 1.2). The perturbations proposed to have had a negative influence on the Broads aquatic ecosystem range from the physical disturbances and diesel contamination from boats (Ellis 1965;Cable 1991) and organochlorine pesticide contamination (Stansfield *et al.* 1989). The extent to which any of these stressors acted individually or in combination is not yet fully known. This is especially true of the role of toxic contaminants. The work of Stansfield *et al.* (1989) demonstrated

clear ecological changes within the Broads studied, but was inconclusive as to the causes, due to the apparent high temporal variation in organochlorine pesticides measured within the sediment cores.

Breakdown of the internal buffering mechanisms that maintain the clear water state is often the starting point of catastrophic plant loss (Moss *et al.* 1996a). Identification of key ecological interactions that maintain such conditions is therefore a prerequisite to determining the mechanism by which shifts between alternative equilibria occur. Predation by fish upon plant-associated grazing invertebrates represents an important trophic interaction that influences the structure of the periphytic grazing community (Bronmark 1989:Jones and Sayer 2003;Liboriussen *et al.* 2005). Therefore variation in the structure of fish communities between shallow lakes, either by chance colonisation, human modifications through stock management (Skov *et al.* 2002) and natural or pollution induced fish kills, represent strong top-down influence on overall ecosystem state (Perrow *et al.* 1997;Lammens 1999).

The examples given in this section and in those focussing on ecotoxicological impacts demonstrate the sensitivity of shallow lake ecosystems to perturbation. In terms of toxicant impacts upon shallow lake equilibrium, the ecotoxicological response of sensitive key structural or functionally important groups, to intense pollution episodes, represents a perturbation that could potentially have ecosystem wide consequences. It is this type of toxicant perturbation to shallow lake ecology that requires further study.

1.3.7 A Broadland perspective

The landscape and hydrology of the Broadland region provides the geographical and physical setting of the present study and is described.

The majority of broads in the river valley floors are currently in direct connection with one of the main rivers that drain the area, namely the Bure, Ant, Thurne, Yare and Waveney (Figure 1.1). Surface connection is via channels, or dykes, originally cut for transport of marsh products, e.g. reed and sedge, which became a locally important economic resource once peat digging had ceased. The low gradient river channels become increasingly brackish as they flow toward the North Sea at Great Yarmouth. Water levels in the lower river reaches are tidally influenced as water

backs-up the river systems at high tide, with occasional saltwater surges penetrating far inland (Clarke 1990). All the broads are <3 m deep with average depth being approximately 1.2 m (Moss 2001). The hydrological catchment areas of the Broadland rivers are predominately arable agriculture, with one major urban area, the city of Norwich, located at the confluence of the Rivers Wensum and Yare. Adjacent to a large proportion of the open water is a landscape of drained marshland and un-reclaimed fen, the area being collectively known as Broadland.

Historically the rural economy that shaped and managed the Broadland region had minimal pollution impact on the diverse and abundant wildlife that flourished in its clean, quiet waterways (George 1992). The local naturalist Ted Ellis commented on the broads being renowned for it's "gin" clear water and prolific submerged plant communities (Ellis 1965), these conditions being common prior to the major decline which began to happen from the 1960s onwards. The unpolluted waterways and swamps provided an incredible diversity of habitats which were home to equally diverse floral and faunal communities. Broadland as a whole is still widely recognised for the richness of its wildlife and unique landscape. International conservation designations reflect the areas special status. "The Broads" SAC (Special Area of Conservation) and "Broadland" SPA (Special Protection Area) have been designated under the respective drivers of the EU Habitats Directive and EU Birds Directive (Broads Authority 2004). Management of the area is now the responsibility of the Broads Authority, and the Norfolk Broads is the only wetland National Park in the UK. Currently only a few broads remote from intensive agriculture and disturbance retain healthy abundances of the macrophyte species which characterised the pre-pollution period in the Broads (Kennison, Dunsford, and Schutten 1998), such as species of stonewort, several of which now are of high conservation value (Stewart and Church 1992).

A large pleasure boat industry and an increasing number of privately owned craft that use the navigable waterways of the Norfolk Broads (Figure 1.3) have been traditionally serviced by a local boatyard industry. Typical maintenance involves the removal of biological encrustation that colonises boat hulls, the so-called biofouling. Such biological growth increases the drag between hull and water, which can lead to decreased fuel efficiency or slower racing speeds during competetive sailing and biofouling can look unsightly on the painted hulls, especially at the waterline.

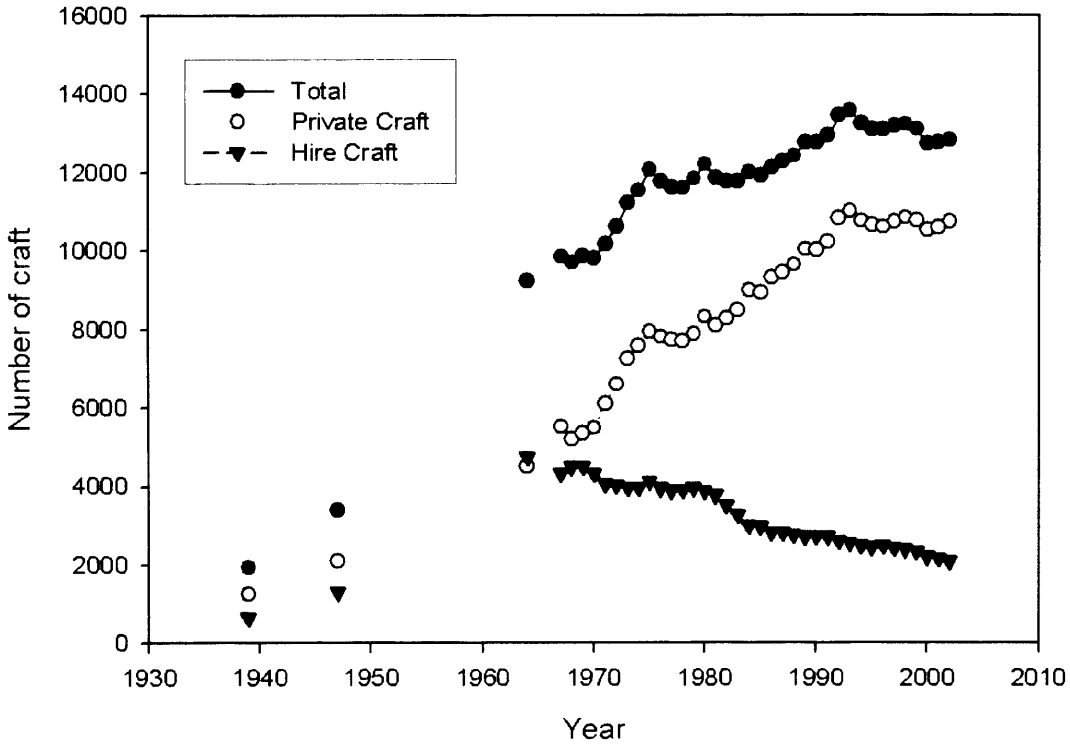

Figure 1.3 Number of boats present in the Broads.
(data from (George 1992;Broads Authority 2004); numbers do not include rowing boats or canoes)

Although originally designed for use at sea, TBT use as an antifoulant was widespread in the area until 1987 (Waite *et al.* 1989). It is thought that the factors influencing boat owners in the Broads to use such antifoulant paints were due to the perceived negative effects of biofouling, little understanding of the potent toxicity and partly the fashionable marketing of such products.

1.3.7.1 Degradation of aquatic vegetation communities

The shallow lakes of the Broads have a long and rich record of natural history inquiry and research (Ellis 1965;George 1992;Moss 2001). This resource is of great benefit for characterisation of the ecological changes that have occurred over the last century.

Early research on the vegetation of Broadland indicated that a natural hydroseral succession in plant communities existed, albeit within a modified wetland landscape, extending from wet fen through to an open water habitat (Pallis 1911).

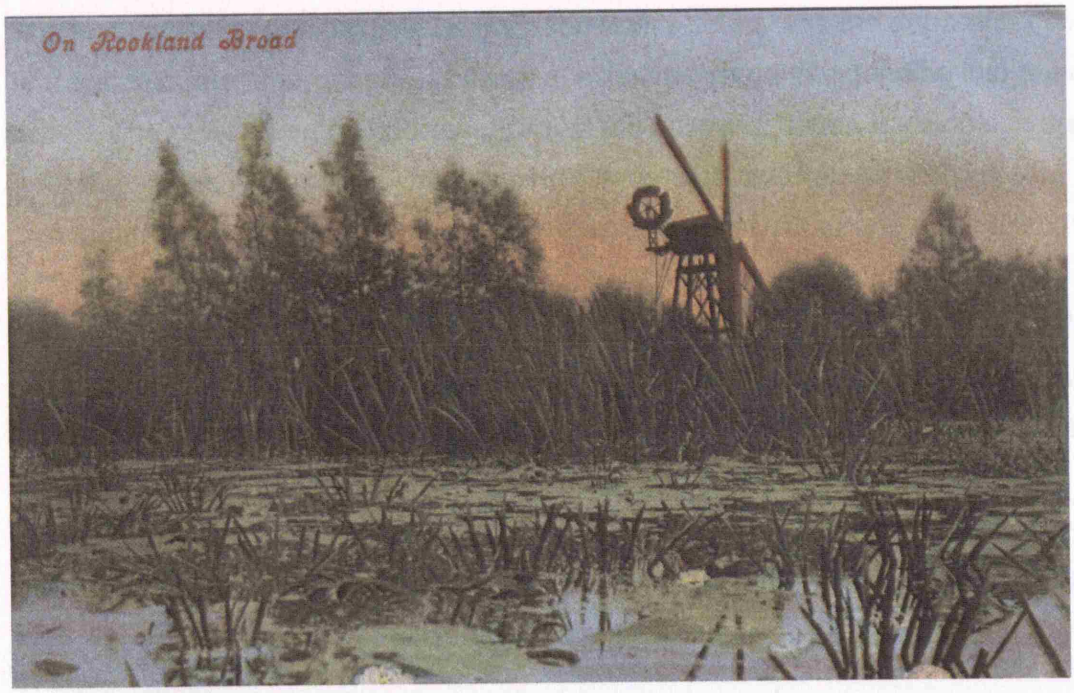

Figure 1.4 Photographic postcard of Rockland Broad on the River Yare in the early 20th century, showing water soldier and white water lily

In the open waters of the broads, a diverse submerged macrophyte community existed, including stoneworts (*Chara* and *Nitella* sp); pondweeds (e.g. *Potamogeton natans* L., *P. lucens* L.); holly-leaved naiad (*Najas marina* L.); bladderwort (*Utricularia vulgaris* L.); and water soldier (*Stratiotes aloides* L.)(Figure 1.4). Alongside these submerged macrophytes, floating leaved and emergent species penetrated into the shallow waters. Floating leaved species consisted of the yellow water lily (*Nuphar lutea* L.), white water lily (*Nymphaea alba* L.) and frogbit (*Hydrocharis morsus-ranae* L.).

Nearer the shore dense stands of common reed (*Phragmites australis* (Cav.) Trin. ex Steud.); the reedmaces (*Typha latifolia* L. and *T. angustifolia* L.) and club-rush (*Schoenoplectus lacustrius* (L.) Pallas); formed the greater part of the littoral plant community, which often extended some distance into the open water as "hover", or floating reed mats. Trees and shrubs capable of withstanding waterlogged soils, such as willows *Salix* sp. and Alder *Alnus glutinosa* (L.) then dominated the marginal areas and higher ground, if traditional local management did not remove the trees to encourage a fen habitat.

The first indication of biological degradation to this scene was the apparent regression of the emergent reedswamp species fringing and extending into the open water. The most common species, *Phragmites australis*, began to decline in area after the 1940s, along with *Typha* sp. and *Schoenoplectus* sp., as interpreted through aerial photography and old O.S. map coverages (Boorman and Fuller 1981). At the time a number of causes were attributed to this decline, including direct physical damage to the macrophytes from coypu and geese grazing and wash from passing motor-boats (Ellis 1965). Pollution effects were also documented as likely causes, ranging from the effects of sewage (George 1992), diesel spills from boats (Cable 1991) and excess nitrates from agricultural fertiliser run-off (Boar, Crook, and Moss 1989). Among these factors, all are likely to have had varying degrees of influence, with the retreat of reedswamp from open water peaking in the mid 1950s in most broads (Boorman, Fuller, and Boar 1979).

Loss of submerged macrophyte communities was also observed from the 1960's (Morgan 1972;Mason and Bryant 1975) and apparently occurred relatively quickly. Jackson (1978) provided a comprehensive review of the status of aquatic macrophytes after much of this former diversity was lost. His findings show that by the 1970s the decline had affected nearly all the lakes in the Norfolk Broads. Most sites by this time had an open water devoid of submerged macrophytes and only a thin band of littoral reed. Water lilies were the only aquatic plant found in most of the River Ant and Bure broads, with even their abundance apparently in decline. Palaeolimnological evidence for the once macrophyte dominated state having turned to one of planktonic based primary production has also been presented for many broads (Osborne & Moss 1977;Moss, Forrest, and Phillips 1979;Moss 1979;Manson 1987;Stansfield *et al.* 1989). In conjunction with the loss of submerged vegetation, declines in abundance and diversity of macro-invertebrate species has also been reported (Mason and Bryant 1974). This change in shallow lake ecology was pervasive throughout the aquatic food-web with impoverishment in the fish community also recorded (Phillips *et al.* 1999) and references therein.

1.3.7.2 The role of boats in the degradation of the Broads

The Norfolk Broads have been synonymous as a famous sailing and pleasure craft venue since the early 1900s (Moss 2001). Tranquil passage through the lengthy navigable waterways, beautiful scenery and the wealth of biological diversity were

major attractions for holidays afloat in the early 1900's. This attraction did not wane, as the increase in total number of craft on the Broads waterways was most rapid during the 1950's (Figure 1.3). A total of 3400 boats were registered in 1947, which increased to around 9200 by 1964 (George 1992).

Figure 1.5 Postcard depicting pleasure boating on Salhouse Broad in the 1950s.

The main focal point for the hire craft industry is the River Bure between the villages of Wroxham and Horning. During the 2003 – 04 boating season, there were around 1700 craft registered as having permanent moorings between the two villages (Broads Authority 2003). However, many more boats from other parts of the open river system are able to pass along this section. The total number of registered craft for the whole of the Norfolk Broads during the same season was over 17500. Boat movement figures taken in August 1998 showed that roughly 900 individual craft passed along the river at points in Wroxham and Horning during a single day (Broads Authority, unpublished data). Given these high boat numbers, not surprisingly, an environmental risk analysis from the usage of AFPs in inland waterways found that the Norfolk Broads was one of the areas most at risk from antifoul biocide contamination in the UK (HSE 2002).

Along the course of the River Bure there are several broads that have a range of hydrological connectivity to the main river channel and navigability to boats. Some are fully navigable and have open access to the river (Figure 1.5). Similarly there

are broads that have a direct hydrological connection with the river but are not open to navigation. These sites are often privately owned or managed nature conservation bodies, for example Natural England manage Hoveton Great Broad and Norfolk Wildlife Trust manage Ranworth Broad. Isolated broads are those located further away from the main river channel, often in small side valleys, which have no open connection to the river, for example Upton and Burntfen Broads (Figure 1.1). These broads are therefore not open to general navigation. This range of connectivity and navigation within the Broads provides a gradient of exposure to the antifouling paint biocides associated with boating activity. Wide spatial variation was found in TBT concentration in this area, around the time of the ban on TBT usage (Waite *et al.* 1989;Dowson *et al.* 1992). However this early work focussed on the navigable broads and river sections, with little information on TBT transport and wider contamination.

1.3.7.3 Water quality and ecological research in the Broads

The recent water quality history of the Norfolk Broads has been well documented, with regular monitoring having been conducted since the late 1970s (Phillips *et al.* 1999). Furthermore, programmes aimed at controlling the impacts of eutrophication and subsequent lake restoration measures have also been conducted in the Broads (Phillips 1984;Moss *et al.* 1986;Moss *et al.* 1988;Phillips and Jackson 1990;Moss *et al.* 1996b;Phillips *et al.* 2005). Several of these studies have used variation in the hydrological connectivity of individual broads as a key structural element in the design of field-based research projects (Moss *et al.* 1996b;Phillips *et al.* 1999).

In the 1970s wastewater treatment discharges were identified as the major source of phosphorus delivered into the Broadland rivers (Moss 1983). After World War II, expansion of the sewerage sytem and growth in recreation and tourism in the Norfolk Broads led to increased environmental pressure on the waterways. Over the same period deep draining and ploughing of reclaimed marshland in the river catchments for arable cropping also provided further potential for nutrient loss from previously un-cropped land. As with many environmental problems, the symptoms of decline in the Broads were not obvious or well documented until the majority of the damage had occurred. Little direct information regarding nutrient concentrations during the 1960s and early 1970s exists. Neither is there detailed documentation of which macrophyte species were lost from the broads, the order in which they were lost or

the rate at which their abundance disappeared, although recent palaeoecological work is starting to fill in the gaps.

Increase in nutrient concentration within individual broads has been inferred from diatom TP transfer functions applied to the sedimentary diatom record (Bennion *et al.* 2001). However, use of such techniques in shallow, productive waters such as the Broads, may substantially over-estimate past TP concentrations. This is because of the high abundance of benthic *Fragilaria* taxa in Broads sedimentary records, which are poor indicators of trophic state (Bennion *et al.* 2005), as they have an indirect relationship with P concentration and are more sensitive to habitat availability (Sayer 2001). There is therefore an apparent gap in data and knowledge of the quantitative ecological changes that occurred during the 1960s loss of macrophytes and which factors may have instigated such a dramatic shift.

1.3.8 The role of palaeolimnology

Requirements for knowledge of past lake ecosystem structure and drivers of change acting therein, means that increasing the temporal extent of biological and chemical datasets beyond the range of contemporary monitoring, is one of the fundamental aims of palaeolimnological reconstructions. This approach has included the determination of temporal profiles within lake sediments of persistent environmental pollutants. Example of toxicants analysed are; heavy metals (Ilyashuk *et al.* 2003;Audry *et al.* 2004;Boyle *et al.* 2004;Nowierski, Dixon, and Borgmann 2006); pesticides (Stansfield *et al.* 1989;Miskimmin *et al.* 1995); PAHs (Quiroz *et al.* 2005;Donahue, Allen, and Schindler 2006); PCBs (Paterson *et al.* 2003;Merilainen *et al.* 2003;Ricking, Koch, and Rotard 2005); and organotins (Müller 1987;Fent *et al.* 1991;Sayer *et al.* 2006). Simultaneous analysis of biological proxies and inference of ecological impacts have also been studied using palaeolimnological approaches. Specific examples include the effects of PCB on algal and chrysophyte communities (Paterson *et al.* 2003); the ecosystem response to historic toxaphene dosing to remove fish (Miskimmin *et al.* 1995); chironomid responses to heavy-metal contamination (Ilyashuk *et al.* 2003); copper and ammonium sulphate discharge from a factory influencing the whole food from algae, zooplankton through to fish (Manca and Comoli 1995); and organochlorine pesticides (Stansfield *et al.* 1989) and TBT (Sayer *et al.* 2006) as potential stressors leading to macrophyte loss in shallow lakes.

The range of direct biological proxies that can be traced within core material from shallow lakes includes quantatative temporal variation in diatoms (Battarbee *et al.* 2001;Sayer and Roberts 2001;Bennion *et al.* 2005); cladocera (Stansfield *et al.* 1989;Brodersen, Whiteside, and Lindegaard 1998;Jeppesen *et al.* 2001); fish (Davidson *et al.* 2003); macroinvertebrates (Hofmann 1983;Brodersen and Lindegaard 1997;Odgaard and Rasmussen 2001); and submerged plants (Birks 1973;Davidson *et al.* 2005). In addition to the analysis of biological remains, geochemical profiles which can indicate changes in ecological processes and productivity include techniques such as analysis of algal fossil pigments (Mackereth 1965;Garcia-Rodriguez *et al.* 2002;Kauppila and Valpola 2003;Waters *et al.* 2005) and stable isotopes of carbon and nitrogen contained in sediment organic matter (Hutchinson and Cowgill 1973;Brenner *et al.* 1999a;Lin, Wu, and Wang 2006). Use of the palaeolimnological approach has been successfully applied to determining the ecological structural changes that have occurred during the switch in alternative equilibria from macrophyte to phytoplankton dominance in shallow lakes (Karst and Smol 2000;Little and Smol 2000;Brodersen *et al.* 2001;Rasmussen and Anderson 2005) and also in determining past ecological conditions within the Broads (Moss *et al.* 1979;Moss 1988;Bennion *et al.* 2001).

Multi-proxy palaeolimnological studies whereby several of these complementary analyses are performed from single cores provides a powerful means of reconstructing past lake conditions and inferring ecological change as a result of human-induced stresses (Engstrom *et al.* 2006;Pienitz, Roberge, and Vincent 2006). In the present study application of multi-proxy techniques across a gradient of exposure to TBT contamination is predicted to generate robust data regarding the response of shallow lake ecosystems to such stress. The present study will build upon the previous work of Sayer *et al.* (2006) which demonstrated that ecological changes in two cores from the Broads were coincident with the onset of TBT contamination.

1.4 Aims

Loss of macrophytes from shallow lakes has been a widely discussed ecological problem (De Nie 1987), with causal factors attributed to perturbations to the ecological structure and functioning which maintains the macrophyte dominated state (Scheffer 1998; Moss *et al.* 1996a). The shallow lakes of the Norfolk Broads, E. England are a classic example of a wetland area that has witnessed extensive ecological change, with ecological stresses arising through intensification of agricultural methods, increased population pressure and need for sewage effluent disposal and also an increased pressure from recreational boating (George 1992; Moss 2001). All broads adjacent to the River Bure, with open navigational access, currently have poor ecological condition in terms of macrophyte populations, with little or no plant growth (Broads Authority 2004). The Broads has had a rich history of environmental and conservation led scientific investigation, especially focussed on mitigating the effects of elevated nutrient loads. Recent palaeolimnological research suggests however, that the once widely used antifoulant biocide TBT, may have been an additional stressor in this freshwater system, capable of precipitating a shift to algal dominance (Sayer *et al.* 2006).

The present study aims (through a spatio-temporal assessment of antifoul biocide concentrations in the River Bure waterway, in conjunction with a palaeolimnological approach) to reconstruct the changing ecology of two shallow lakes, and determine whether TBT could have been a contributing factor in the switch from macrophyte to algal-dominated state. Analysis of water and surface sediments for contemporary antifoul biocide concentrations will be carried out to improve understanding of the transport mechanisms that would have been responsible for dilution and dispersion of TBT.

Within the River Bure system, Wroxham Broad was previously found to be the most TBT contaminated site outside of boatyards (Waite *et al.* 1989). Sampling TBT concentrations, and its degradation products, in contemporary sediments from a variety of locations in the River Bure waterway, aims to reveal the spatial variability in relation to each sampling locations exposure to boating activity. There are also a range of environmental, hydrological and biocide specific physio-chemical factors that influence the environmental fate and distribution of boat-derived contaminants once they have been released into the aquatic environment (Hoch 2001;Voulvoulis,

Scrimshaw, and Lester 2002). As such, sampling at the river scale will enable determination of the presence of any contamination gradient of antifoul biocides, especially between broads with varying exposure to recreational boating. The relationship between the spatial variabilty of the TBT contamination and that of the modern organic booster biocides will be evaluated to see how similar the contamination pattern is between the two biocide tyoes. As TBT inputs ceased in the Broads after the 1987 ban, contemporary sampling of the modern organic antifoulant biocides (e.g. Irgarol 1051 and diuron), is required to determine the existing hydrological processes and sediment characteristics that exert an influence over the spatial distribution of such boat-derived biocides. This will be determined through widespread sampling at the river scale and repeated through quarterly sampling through a whole season. In addition, intensive sampling of AFP biocide distribution within water and sediment of a single broad will help determine the dominant in-lake transportation processes. This work will be valuable for understanding of contaminant fate and transport in the Norfolk Broads and will give insights into how AFP biocide contamination varies in relation to the level and type of boating activity in freshwater systems.

The present study also aims to extend and further develop a palaeolimnological approach in determining whether ecological changes occurred at the onset of TBT contamination in other broads connected to the River Bure. Multi-proxy techniques will be adopted to give several lines of evidence for ecosystem wide changes associated with the loss of macrophytes from such shallow lakes. Sampling further broads associated with the River Bure aims to determine whether the results from the Wroxham Broad study (Sayer *et al.* 2006) are an isolated occurrence, or part of a wider, repeated pattern within the system. The present study also aims to provide an analysis of the existing data to evaluate the potential that TBT may have in causing ecological effects in shallow lakes. It is hoped that the combination of determining contemporary biocide concentrations and the historical deposition recorded in sediment cores will produce a powerful tool with which to assess both temporal and spatial variation in environmental contamination within complex freshwater wetlands.

CHAPTER 2 – STUDY DESIGN AND METHODS

2.1 Study area

Within the Broads as a whole, the most popular part of the navigable waterway, in terms of pleasure boat movements and total numbers of registered moorings is the River Bure. Figure 2.1 shows the number of registered boats in 2003 which were >4.52 m in length. This minimum hull length has been used by the Environment Agency when calculating numbers of craft coated with antifoul paint. For boats below this length the practice of antifouling has been found to be uneconomic and not commonly performed (F. Fiorentina, pers comm.).

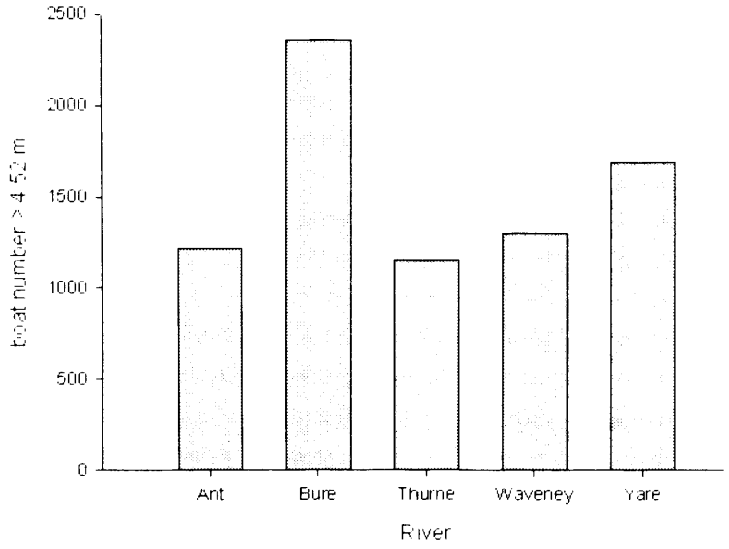

Figure 2.1 Total boat numbers >4.52 m registered on the Broadland rivers, summer 2003.

The River Bure and its associated broads have also been shown to be contaminated by both TBT (Waite *et al.* 1989;Dowson *et al.* 1992;MAFF 1993;Dowson *et al.* 1994) and organic antifouling biocides (Lambert *et al.* 2006). Due to the high exposure to boating activity and history of AFP biocide contamination, the River Bure was therefore selected as the focal area for the present study. Previous research has also helped inform the location of sample sites, as extensive contamination of AFP biocides has been shown to exist in the area, with boatyards being most contaminated (Waite *et al.* 1989;Dowson *et al.* 1994).

The River Bure and its associated broads provide an appropriate study area for a number of reasons. For example, abiotic factors such as climate, geology and land-use are relatively consistent within the most popular and picturesque section of the navigable river, which extends from the upstream navigational limit at Horstead Mill to the most downstream navigable broad, South Walsham Broad (see Figure 2.2).

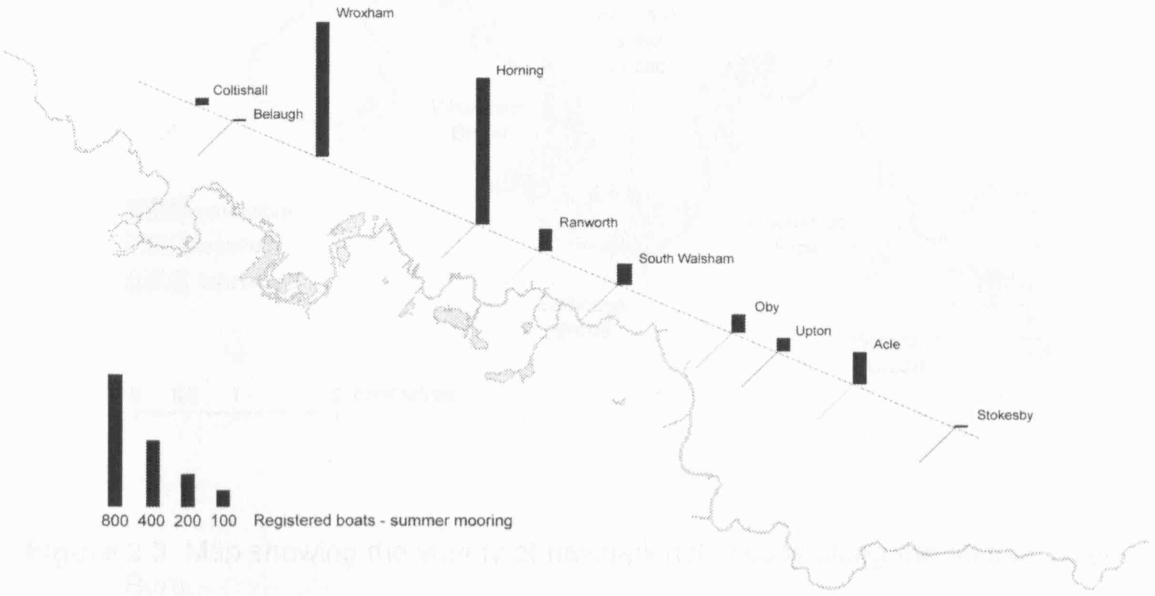

Figure 2.2 Number of registered boats >4.52 m length along the River Bure. Data for summer 2003 (Broads Authority 2003).

The major difference between the individual shallow lakes, in terms of their exposure to antifoul biocides, is their hydrological connectivity to the main river channel, and whether they are navigable to boat traffic. The broads fall into one of three categories. They are either, fully open to navigation (navigable); hydrologically connected to the waterway, but not open to navigation (connected); or have no surface water connection and are consequently not navigable (isolated). Figure 2.3 displays this variation in exposure to boating activity along the River Bure waterway.

There is also variation in the degree of hydrological connectivity to the main river channel between the navigable and connected broads. This factor influences the flushing rate or water residence time within each broad, which may be important for biocide transport.

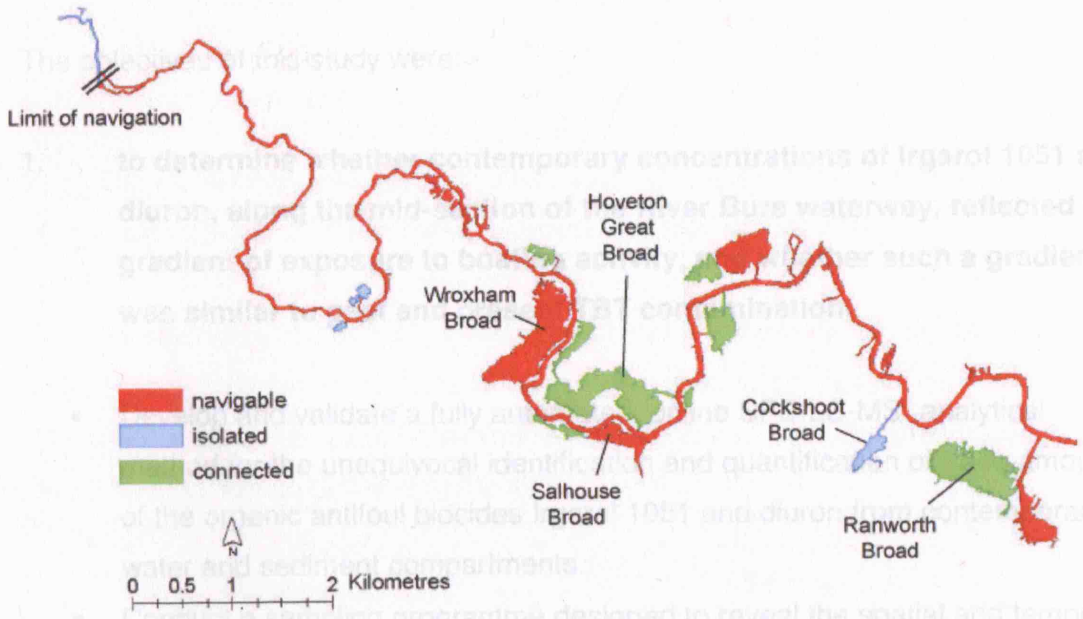

Figure 2.3 Map showing the variety of navigational access along the middle River Bure.

For example Wroxham and Salhouse Great Broad both have two openings into the main river, whereas Ranworth Broad has only one. A value of 82 days has been measured for the retention time of Wroxham Broad (Moss *et al.* 1989). At the lower end of the river valleys, broads such as Ranworth and South Walsham, are over 600 m away from the current river channel, which is predicted to increase their retention times.

2.2 General approach

2.2.1 Objectives

The objectives of this study were: -

1. **to determine whether contemporary concentrations of Irgarol 1051 and diuron, along the mid-section of the River Bure waterway, reflected a gradient of exposure to boating activity, and whether such a gradient was similar to past and present TBT contamination.**

- Develop and validate a fully automated, online SPE-LC-MS^n analytical method for the unequivocal identification and quantification of trace amounts of the organic antifoul biocides Irgarol 1051 and diuron from contemporary water and sediment compartments.
- Conduct a sampling programme designed to reveal the spatial and temporal variation of antifoul biocide concentrations across a range of sites with different navigational access and boating activities.
- Compare the spatial distribution of persistent TBT surface sediment contamination with that of modern organic antifoul biocides, to identify whether a similar contamination gradient existed, despite differences in physio-chemical properties of the two biocide types.
- Collect multiple contemporary environmental samples within a single connected broad to determine the spatial variability (to give confidence in using one sample per sample site in the wider survey) and identify the major influences on antifoul biocide distribution within that lake.

2. **to establish, using palaeolimnological techniques, whether past ecological changes in broads with differing exposures to boating activity, were synchronous with the initial period of TBT contamination.**

- Perform multi-proxy palaeolimnological analysis on sediment cores collected from broads with differing exposures to boating activity.
- Quantify the TBT pollution history profiles from radiometrically dated sediment cores.

- Quantify the variation in biological proxy data from a range of aquatic organism groups.
- Statistically analyse of the biological proxy data to determine whether ecological change occurred in synchrony with the onset of TBT contamination within the core profile.

3. to determine whether the effects of TBT contamination can be distinguished from those caused by other stressors.

- Establish whether TBT concentration levels were sufficient to cause the biological changes inferred from the palaeoecological record.
- Analyse cores for geochemical sigatures able to demonstrate change in trophic status of the waterbodies.

2.2.2 Introduction

To determine relative levels of environmental exposure from AFP biocides within the study area, including the period before the introduction of TBT, a sampling programme that was capable of generating contaminant data within a space-time framework was followed. Spatial variation of organic antifoul biocides in water and surface sediments, as well as the persisting concentrations of TBT in surface sediments, were determined through regular sampling. This was complemented by measuring temporal variation in TBT contamination, over a decadal time scale, using palaeolimnological techniques at two shallow lake sites in the River Bure catchment. Through a combination of these two approaches, the study aimed to determine relative AFP biocide contamination both spatially and historically. From these data, inferences were then made possible, regarding antifoul biocide environmental fate and transport within the study area. The influence of such fate processes upon TBT distribution are important for the interpretation of relative variation in TBT concentrations between separate core profiles. With a clearly established picture of the extent and degree of TBT contamination within the River Bure waterway, the ecological changes observed in sediment cores could then be analysed in terms of the potential impact of toxicity from TBT.

2.2.3 Contemporary biocide sampling site selection

More detailed analysis of the Broads Authority boat registration data showed that the boatyard complexes within the villages of Wroxham and Horning were the major hubs of boating activity on the River Bure (Figure 2.2). In a study of TBT contamination along this stretch of the navigable Bure in 1986, Waite et al (1989) found that Wroxham Broad had the greatest soluble TBT concentration outside of boatyard areas. More recently surface sediment samples collected in 2000 showed that TBT was still detectable at various sites along the River Bure waterway (Sayer, unpublished data). To determine whether widespread measurable concentrations of TBT would be encountered along this stretch of river, a pilot survey of TBT surface sediment concentrations was conducted in April 2003. At the same time, water samples were also collected for analysis of Irgarol and diuron, the contemporary organic AFP biocides. This initial sampling focussed on the busiest boating areas of the River Bure that coincided with those areas reported as being most heavily contaminated with TBT in the past. For these reasons, 15 sites between the navigational limit at Horstead Mill and the second most downstream broad, Ranworth Broad were selected, covering an approximate distance of 20 kilometres by river. Given the variation in navigational access, as shown in Figure 2.3, the aim was to determine if a TBT contamination gradient existed with decreasing concentrations between boatyard, navigable broads and connected broads, with an isolated broad as a non-exposed control site. Such a sampling programme would determine the spatial distribution of AFP biocide contamination and whether this extended into non-boated waters. See Figure 2.4 for pilot and subsequent quarterly sample site locations.

Following previous antifoul biocide studies along this river, a sample site above the limit of navigation was chosen, in order to detect whether there were any alternative sources of AFP biocides arising from within the catchment. This site upstream (u/s) of Horstead Mill, was in a shallow, non-tidal reach of the river, where a lock and weir system has prevented navigational access throughout the period of TBT usage. Also sampled were four navigable mid-channel river sites equally spaced along the waterway; one broad isolated from the river (Cockshoot); two connected broads (Hoveton Great, Ranworth); two navigable broads (Wroxham, Salhouse); and five boatyards. See Table 2.1 and Figure 2.4 for location map and site details.

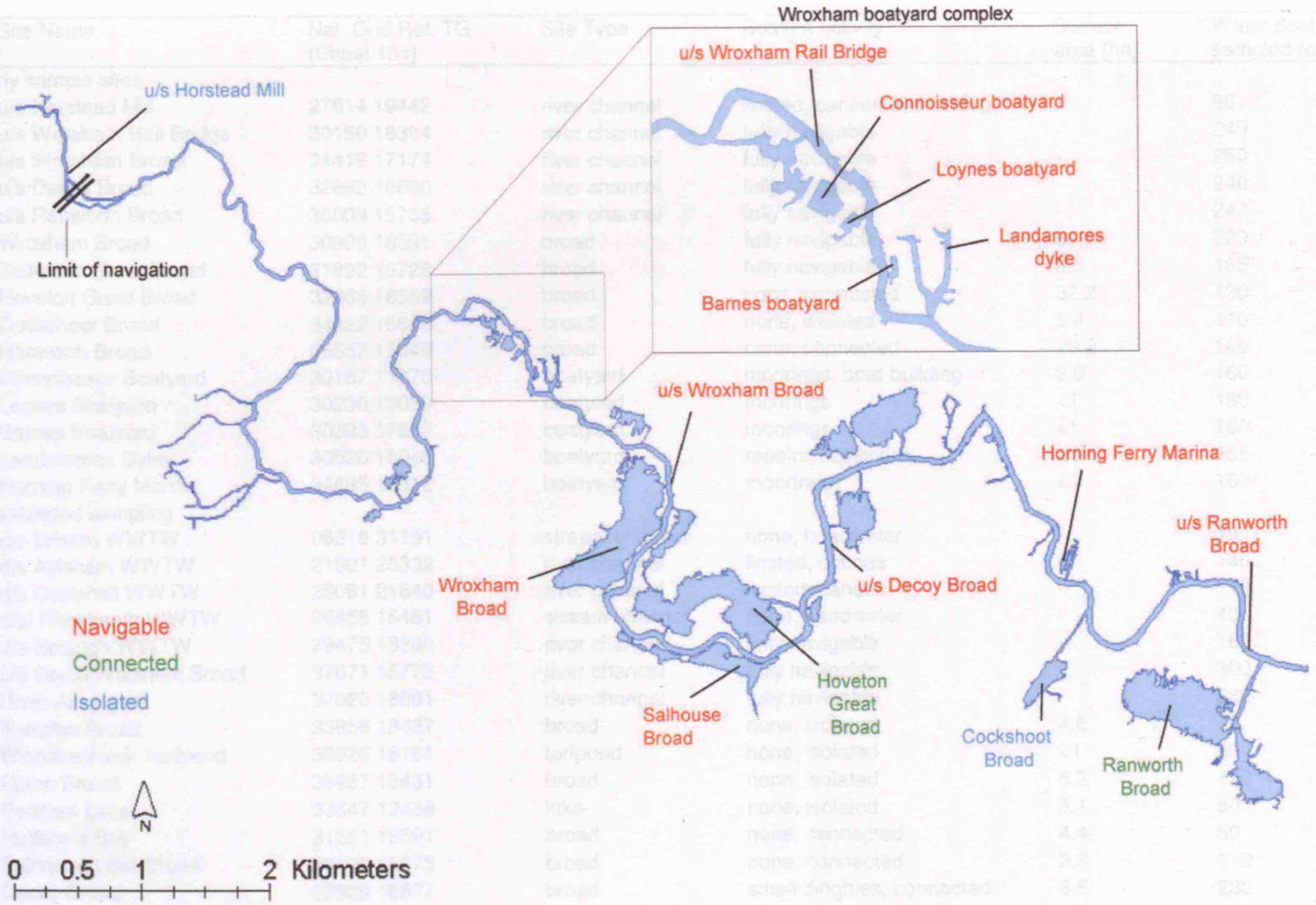

Figure 2.4 Location map of the pilot and subsequent quarterly sampling sites along the River Bure.

Table 2.1 Sample site location and details

	Site Name	Nat. Grid Ref. TG (Sheet 134)	Site Type	Boating activity	Surface area (ha)	Water depth sampled (cm)
Pilot & quarterly sample sites						
	u/s Horstead Mill	27614 19442	river channel	limited, canoes and rowing	-	90
	u/s Wroxham Rail Bridge	30150 18384	river channel	fully navigable	-	240
	u/s Wroxham Broad	31419 17174	river channel	fully navigable	-	260
	u/s Decoy Broad	32662 16690	river channel	fully navigable	-	240
	u/s Ranworth Broad	36009 15755	river channel	fully navigable	-	240
	Wroxham Broad	30999 16591	broad	fully navigable	34.3	220
	Salhouse Great Broad	31892 15726	broad	fully navigable	8.5	165
	Hoveton Great Broad	32066 16559	broad	none, connected	32.2	130
	Cockshoot Broad	34422 15640	broad	none, isolated	5.4	110
	Ranworth Broad	35557 15340	broad	none, connected	29.2	160
	Connoisseur Boatyard	30187 11870	boatyard	moorings, boat building	2.0	160
	Loynes Boatyard	30230 18039	boatyard	moorings	<1	180
	Barnes Boatyard	30393 17862	boatyard	moorings	<1	180
	Landamores Dyke	30520 18048	boatyard	repairs, repainting	<1	165
	Horning Ferry Marina	34495 16512	boatyard	moorings	<1	180
August 2004 extended sampling						
	d/s Briston WWTW	08218 31191	stream channel	none, headwater	-	15
	d/s Aylsham WWTW	21601 25332	river channel	limited, canoes	-	140
	d/s Coltishall WWTW	25061 21640	river channel	limited, canoes	-	110
	d/s/ Rackheath WWTW	26858 15481	stream channel	none, headwater	-	40
	d/s Belaugh WWTW	29475 18380	river channel	fully navigable	-	160
	u/s South Walsham Broad	37671 15775	river channel	fully navigable	-	300
	River Ant mouth	37620 16081	river channel	fully navigable	-	280
	Burntfen Broad	33858 18437	broad	none, isolated	4.6	70
	Woodbastwick Turfpond	33926 16181	turfpond	none, isolated	<1	50
	Upton Broad	38987 13431	broad	none, isolated	5.3	130
	Pedham Lake	33847 12438	lake	none, isolated	3.1	80
	Hudson's Bay	31351 16591	broad	none, connected	4.4	50
	Salhouse Little Broad	32025 15675	broad	none, connected	2.3	110
	Decoy Broad	32929 16877	broad	small dinghies, connected	8.5	230
	Hoveton Little Broad	33147 17600	broad	fully navigable	15.2	170
	Malthouse Broad	35926 14880	broad	fully navigable	10.0	190
	South Walsham Broad	37153 14182	broad	fully navigable	21.2	170

The isolated lake site, Cockshoot Broad, had a surface water connection to the river channel prior to 1980, but a dam was placed across the connecting dyke as part of a lake restoration programme that included sediment removal and is now closed to navigation (Holzer *et al.* 1997). Water from the river does not usually enter the broad, so the site was selected as a control lake for AFP contemporary biocide contamination, being not impacted by current boating activities or surface water movements.

The two connected broads sampled, Hoveton Great Broad and Ranworth Broad, both have surface water connections to the navigable river channel, but boats are not allowed on them, as they are managed as nature reserves. Sampling at these sites aimed to determine whether transport of AFP biocides was occurring from the source navigable areas. In the relative absence of historic data for soluble TBT from many connected broads, contemporary data for the organic antifoul biocides was collected to give an indication of the likely spatial extent of such boat-derived contamination.

Table 2.2 Date and number of organic biocide samples collected from different aquatic compartments.

	Date	TBT	Organic biocides	
		Sediment	Sediment	Water
2003	3rd & 5th Apr	15	-	15
	6th – 7th Aug	-	-	15
	9th – 10th Nov	-	-	15
2004	1st – 3rd Feb	-	-	15
	2nd – 3rd May	-	-	15
	9th – 13th Aug	32	32	32

The two navigable broads regularly sampled, Wroxham and Salhouse Great are downstream of the Wroxham boatyard complex. Four of the boatyard sites sampled were located within this complex, whilst another boatyard was sampled at Horning, a further 5.5 kilometres downstream. Several of the sites chosen for this study had been sampled for organotins in previous studies. This pilot sampling programme was specifically conducted to reveal any gradient in AFP biocide contamination resulting from the variation in navigational access and boating activities between

sites. Determination of the environmental persistence of TBT was also possible through comparison with data from previous studies.

The pilot survey sites were revisited in August 2003 and subsequently sampled quarterly through to August 2004 to determine the temporal variation in organic biocide concentrations over the course of a whole boating season. Table 2.2 gives the dates of sample collection. Boat numbers on the water and total boat movements within the Broads waterways have been shown to vary with season (Hilton and Phillips 1982). From results presented in Hilton & Phillips (1982) boat traffic appears to start in April, with a peak in summer occurring from June to September, declining through October, with relatively little boat traffic during the winter months. This pattern of boat usage on the waterway has guided the timing of sampling occasions.

In August 2004 the range and number of sampling sites was increased, with 17 further sites sampled in addition to the 15 quarterly sample sites. This expanded survey aimed to determine a wider and more detailed picture of the spatial variation in antifoul biocide concentrations across the study area. The bottom half of Table 2.1 gives locations and details of the extra sites sampled. An increased number of broads was selected to give a total of five for each of the navigable, connected and isolated site types (see the coloured labelled sites in Figure 2.5). Two extra river mid-channel sites were sampled, at u/s South Walsham Broad, the most downstream river site sampled and another at the River Ant mouth, where it flows into the River Bure. Sampling this latter site allowed inputs from another popular navigable river to be determined, as inputs from the River Ant may contribute to AFP biocide concentrations observed in the lower Bure.

In addition, five sample sites directly downstream of wastewater treatment works (WWTW) discharges were included in August 2004 (see Figure 2.6 for locations), as detectable concentrations of organotins and diuron have been reported in surface waters from this type of input (Nitschke and Schussler 1998;Stangroom, Collins, and Lester 1998;Gerecke *et al.* 2002). The increased number of sampling sites therefore extended the overall study area further downstream, included potential biocide inputs from WWTWs in the headwaters and increased the sampling intensity within each navigation site type, ie, boatyards; navigable; and connected and isolated broads.

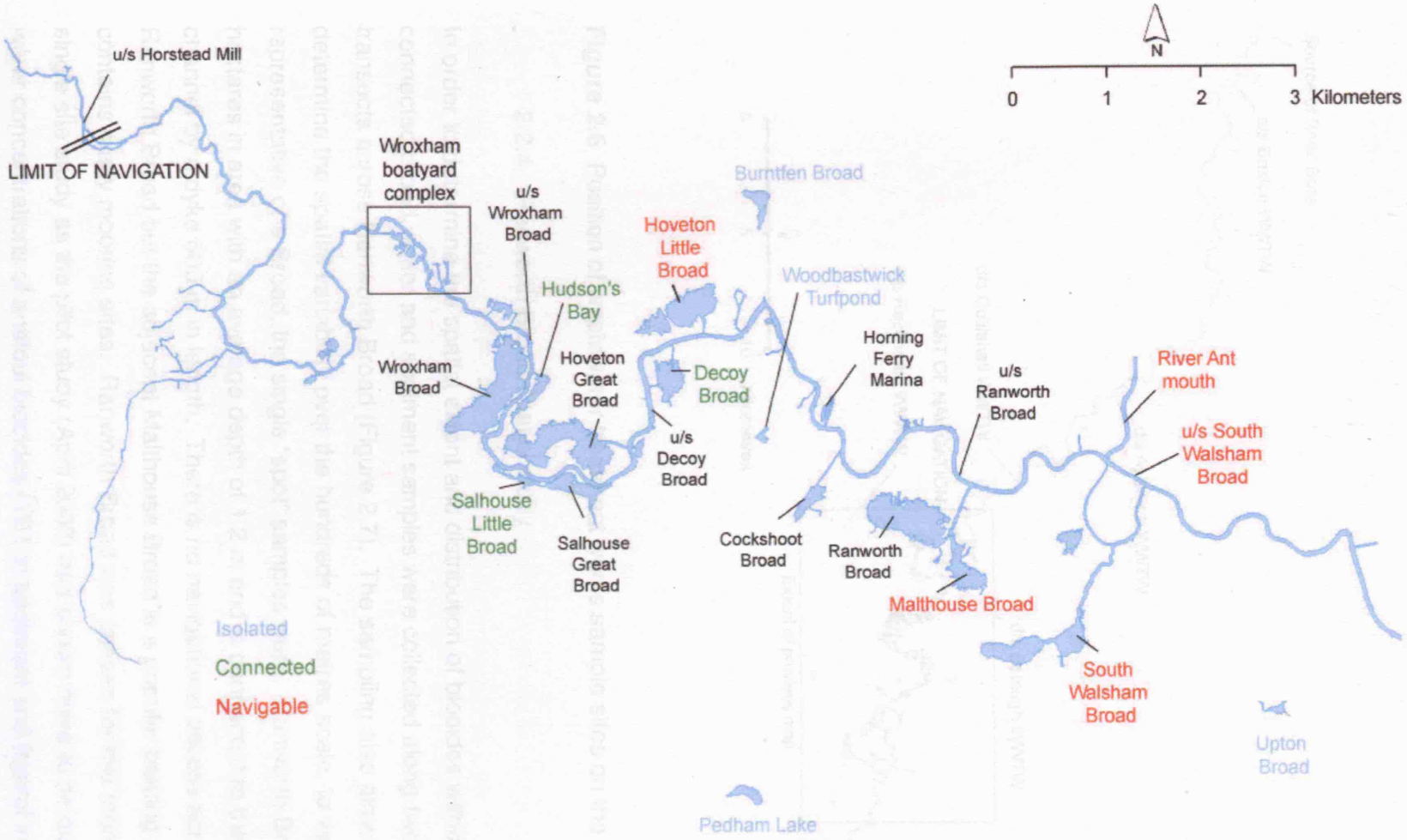

Figure 2.5 Expanded August 2004 sampling – extra site locations in coloured text (not including WWTWs).

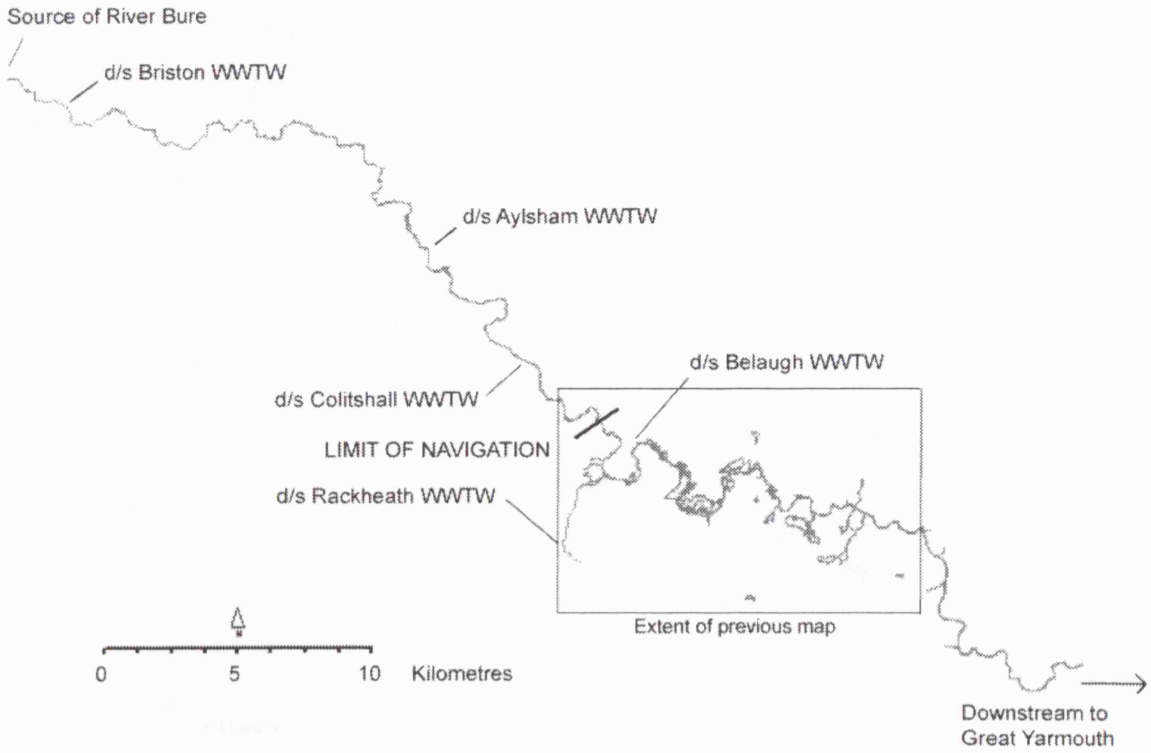

Figure 2.6 Position of wastewater treatment works sample sites on the River Bure

2.2.4 Ranworth Broad spatial study

In order to determine the spatial extent and distribution of biocides within a single connected broad, water and sediment samples were collected along two parallel transects across Ranworth Broad (Figure 2.7). The sampling also aimed to determine the spatial variability over the hundreds of metres scale, to verify how representative of a Broad, the single "spot" samples were. Ranworth Broad is 29.2 hectares in area with an average depth of 1.2 m and is connected to the main river channel by a dyke 600 m in length. There is no navigational access across Ranworth Broad but the adjacent Malthouse Broad is a popular boating area and contains many mooring sites. Ranworth Broad was chosen for this more detailed, single site study as the pilot study (April 2003) had shown there to be quantifiable water concentrations of antifoul biocides (TBT in sediment and Irgarol in water samples) within this site despite it having no direct exposure from boats on its surface. The sites hydrological connection to the navigable system is also relatively

simple, compared to other broads, which often have multiple connecting channels. As Ranworth Broad is in the lower reaches of the River Bure it is subject to tidal water-level fluctuations and occasional saline intrusion (Pitt, Kelly, and Phillips 1997).

Figure 2.7 Biocide sampling transects taken across Ranworth Broad

Sampling was carried out on 10th August 2004 with 5 sample points positioned along each transect, which extended from where the connecting dyke joins the broad, across to the opposite, western side. At each of the ten sample points, water samples were analysed for organic biocides as detailed in Chapter 3. Sediment samples were analysed for organic biocides and organotins, as well as characterisation of the particle size distribution and organic matter content. These latter parameters are important variables that can influence the amount of sorption by TBT and organic biocides to sediments (Gao *et al.* 1997;Hoch and Schwesig 2004). Results are presented in Section 5.3.

2.2.5 Palaeolimnological sample sites

The initial site for palaeolimnological study was Salhouse Great Broad (core code SALG1), a medium-sized, navigable broad, one kilometre downstream of Wroxham Broad. This site was predicted to have high levels of TBT and a contamination

profile similar to that found in Wroxham Broad (Jackson 2003), given its open access to navigation and relatively short distance downstream of the Wroxham boatyard complex, approximately 2.5 kilometres by river.

From the limited historical evidence available, it appears that the broad once contained macrophyte species including *Ceratophyllum demersum* and *Potomogeton lucens* (data from files held at English Nature, Norwich) and old photographic evidence shows stands of water-lilies and possibly other unidentified floating-leaved macrophytes around the margins, see highlighted areas in Figure 2.8. A survey conducted in 1968 found no aquatic macrophytes in the broad (data from files held at English Nature, Norwich). The broad's current status in terms of submerged macrophyte growth is still very poor, with little or no growth recorded in the last decade (Broads Authority, unpublished data) and it generally has turbid water. Core SALG1 was collected on 04/03/2003 from the western basin of Salhouse Great Broad (52° 41.394' N, 1° 25.655 E)(Figures 2.8 and 2.10 for core location). The water depth at the core location was 160 cm.

The second lake selected for palaeolimnological study was Hoveton Great Broad (core code HGBO1), a connected broad, situated on the opposite, northern side of the river channel to Salhouse Great Broad. This site was chosen due to its close proximity to Salhouse Great Broad and contrasting exposure to boating activities. These characteristics were considered important to enable comparison of the ecological changes at the time of first TBT usage between sites with differing TBT exposure. The available aquatic macrophyte records and aerial photographic evidence indicated that extensive and diverse macrophyte beds were present in the broad at least up until the early 1960s (Jackson 1978).

Species recorded include *Stratiotes aloides*, *Ceratophyllum demersum*, *Lemna minor*, *Lemna trisulca*, *Myriophyllum verticillatum*, *Nuphar lutea* and *Nymphaea alba* (Lambert and Jennings 1951;Morgan 1972). However surveys conducted in the late 1960s and 70s found much of the former diversity and abundance had disappeared, with only reduced stands of water lilies and sparse populations of *C. demersum* and *Potamogeton crispus* (Morgan 1972;Mason and Bryant 1975;Jackson 1978). The broads current ecological status in terms of aquatic macrophyte growth is poor, with little or no plant populations and turbid water, although the connected Hudson's Bay does have stands of waterlilies (Broads Authority 2004).

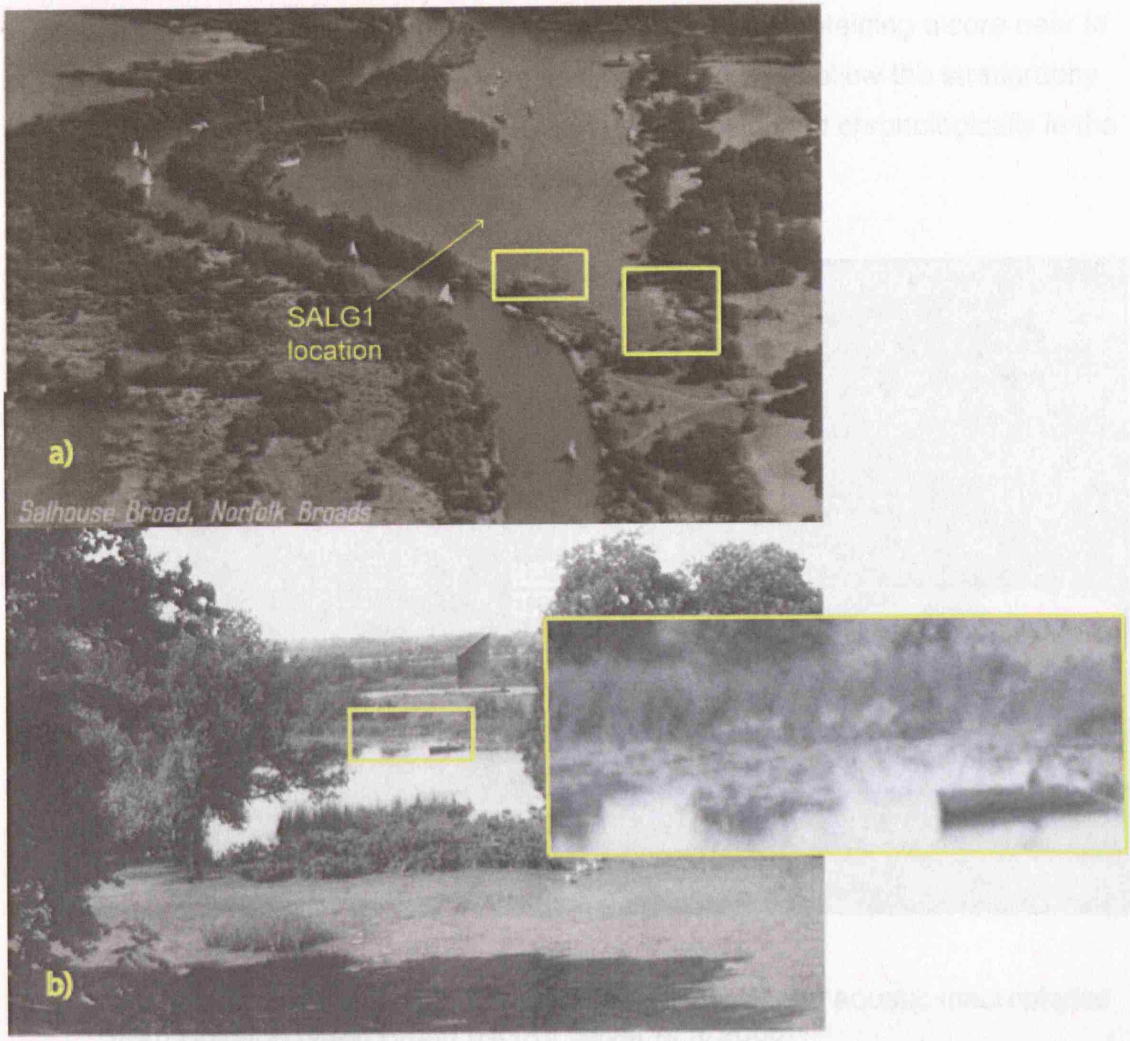

Figure 2.8 Photographs of Salhouse Great Broad a) 1950s-60s, b) 1902, with floating-leaved macrophytes, probably waterlilies (highlighted) and location of SALG1 core.

Core HGBO1 was collected on 14/01/2005 from much closer to the lake margin than SALG1. Photographic evidence (see Figure 2.9) showed that this was an area where distinct regression of the littoral vegetation had occurred between the 1946 and 1969 pictures. The HGBO1 location (52° 41.374' N, 1° 25.337 E) was about 20 m out from the current south margin of the central basin in a water depth of 120 cm.

A second core, code HGBO2 (52° 41.376' N, 1° 25.338 E) was taken on the same day with a standard 7.4 cm I.D. Livingston corer. This core was collected approximately 5 m away from HGBO1, some 20 m from the lake margin (Figure 2.10 for core locations). Use of the longer thinner tube Livingston corer enabled the entire

sequence of the lake sediment to be collected, as the wide diameter corer had an operational sediment sampling depth no greater than 1 m. Obtaining a core near to HGBO1, extending down to the pre lake sediments, aimed to allow the stratigraphy observed in the wider diameter core (HGBO1) to be positioned chronologically in the context of the entire sediment sequence.

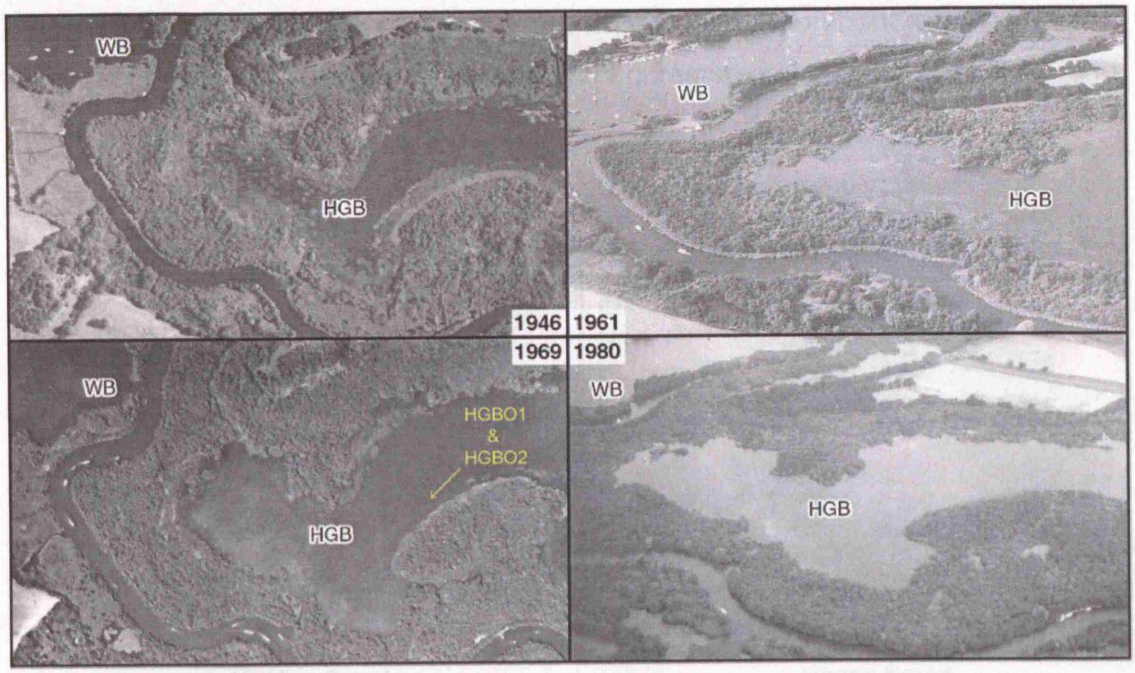

Figure 2.9 Aerial photography showing the loss of littoral and aquatic macrophytes from Hoveton Great Broad (HGB)(Sayer *et al* 2006)
(The navigable River Bure and Wroxham Broad (WB) are also visible in each picture)

The opportunity also arose during the course of this research to analyse TBT contamination from a core taken from Hickling Broad, a navigable broad in the River Thurne catchment, itself a tributary of the River Bure (Figure 1.1 for location of Hickling Broad). A previous study on this core (HICK1) showed there to be distinct changes in the diatom flora at 12 cm depth (Liptrot 2002). Sediment samples had been frozen at the time of collection, and sufficient sediment material remained to perform macrofossil analysis. The core was collected on 27/04/2002 from the Heigham Corner area of Hickling Broad (52° 43.498' N, 1° 35.379 E), in the centre of the basin, approximately 500 m from the main navigation channel.

TBT analysis was also made possible on frozen samples from a core collected from Barton Broad (BART9). Barton Broad is located along the course of the River Ant,

again a tributary of the River Bure (see Figure 1.1 for location map). This core was collected on 10/09/2001 from the Neatishead Arm area of the broad (52° 44.201' N, 1° 29.139' E). The core was collected from a small pocket of undisturbed sediment remaining in the broad, as a restoration project that involved suction-dredging the surface sediments from the entire site, was nearly complete. This meant that the core location was sub-optimal in terms of palaeolimnological study, as it was very close (10 m) to an exposed marginal zone, where wave action/boat wash was evident. Details of all the core collection procedures are given in sections 2.4.1 and 2.4.2.

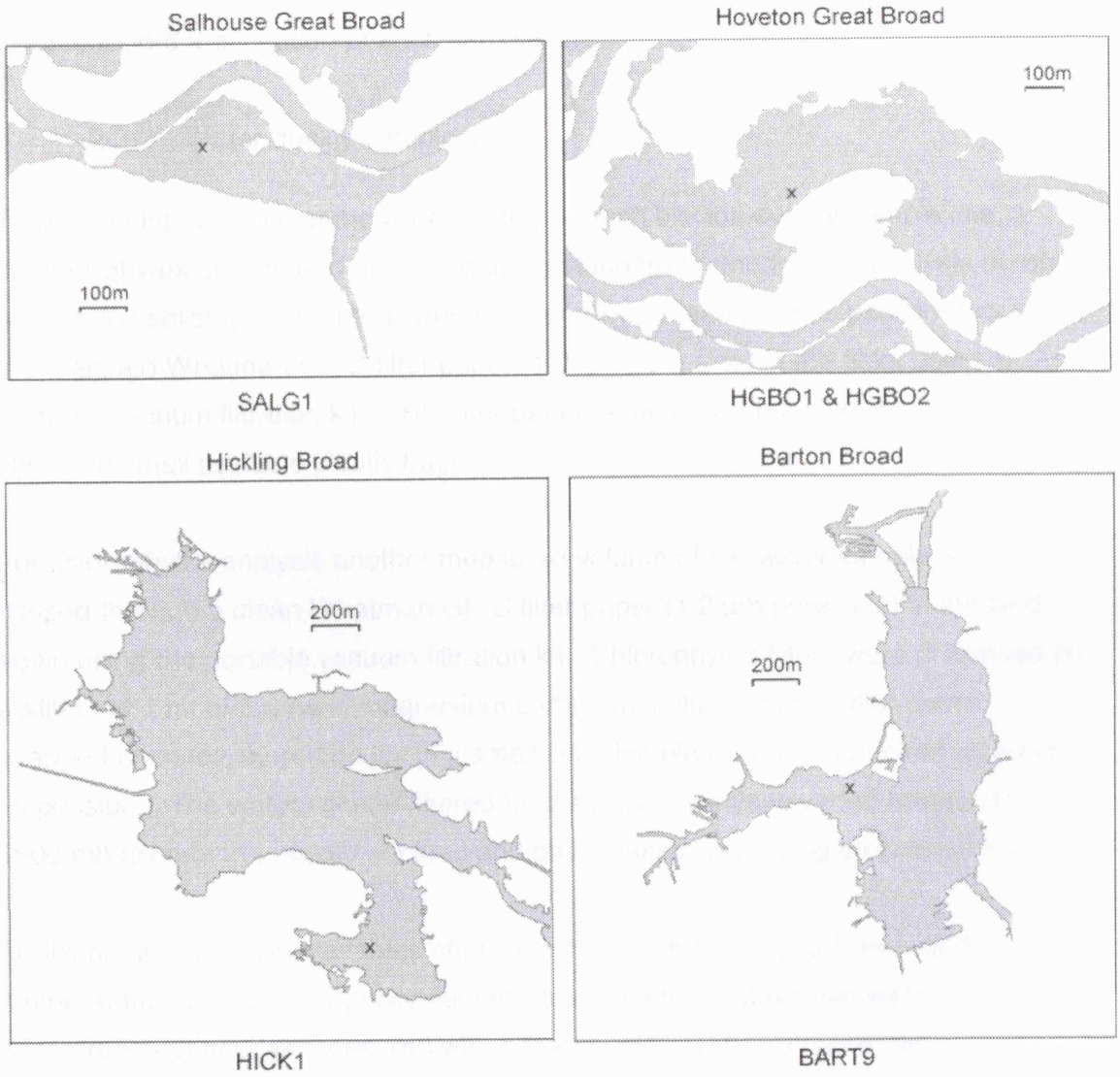

Figure 2.10 Location of cores analysed for TBT.

2.3 Field methods - contemporary environmental sampling

2.3.1 Water sampling for organic biocide analysis

Pre-cleaned 500 ml amber glass bottles were filled at least 20 cm below the water surface at each site and sealed with a foil-lined lid. Pre-cleaning of sample bottles involved soaking in 5% Decon solution overnight, rinsing with distilled water and finally rinsing with acetonitrile before leaving to air dry. After collection the full sample bottles were stored on ice in the dark and refrigerated to <4 °C as quickly as possible. Where sampling could not be conducted from the waters edge, a non-antifoul painted boat was used. A hand-held Global Positioning System (GPS) was used to record the location of each sample site.

2.3.2 Water quality sampling

To provide information on the water quality at each biocide survey sample site, a number of variables were determined using standard methods. For analysis of total suspended solids (TSS) a measured volume of water sample was passed through a pre-weighed Whatman GF/C filter paper (1.2 µm pore size) in the field, using a portable vacuum filtration kit. TSS filter paper samples were folded for storage and placed in small sealable plastic bags.

For chlorophyll *a* analysis another measured volume of the water sample was passed through a clean Whatman GF/C filter paper (1.2 µm pore size) in the field, again using the portable vacuum filtration kit. Chlorophyll *a* filters were preserved by addition of 1 ml of 5% (w/v) magnesium carbonate solution to the filter paper, wrapped in tin foil, stored on ice in a small sealable plastic bag and frozen as soon as possible. The water volume filtered for these two analyses varied (range 200 – 1500 ml) according to sediment load and phytoplankton standing crop at each site.

Additional water quality variables of pH, electrical conductivity (µS cm^{-1}) and temperature were taken with portable electronic meters. Maximum water depth and water transparency, as measured with a Secchi disc, were also recorded.

2.3.3 Surface sediment sampling

Samples of the surface 0-1 cm sediment layer at each site were collected with a 7 cm I.D. Glew gravity corer (Glew 1991). Several individual cores were collected within a 5 m radius to obtain a representative sample from each location. The sediment was carefully extruded to preseve an intact surface sediment layer with excess water pipetted off prior to sub-sampling. For biocide analysis two ~20 cm^3 sub-samples of the bulked sediment were stored in 30 ml glass vials and preserved on ice, in the dark, until frozen to -20 °C upon return to the laboratory. The remaining material to be used for sediment characterisation was placed in a sealable plastic bag and stored in the dark at <4 °C.

2.4 Field methods - palaeolimnological sampling

2.4.1 Large diameter piston corer

In order to collect sufficient sediment for multi-proxy palaeolimnological analyses from a single core, a novel wide diameter version Piston corer was designed and constructed by collegues from the Environmental Change Research Centre (ECRC), University College London. The fieldwork for the present study represented the initial field testing of this new corer.

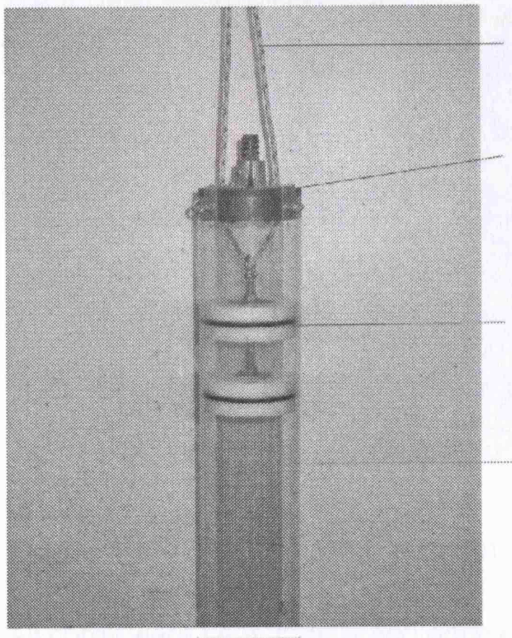

Figure 2.11 The wide diameter "Big Ben" piston corer.

The 14 cm I.D. Perspex core tube was calculated to collect approximately 150 cm^3 of sediment from each 1 cm section upon extrusion. This amount is nearly four times the sediment volume extruded in a 1 cm section from a standard 7 cm I.D. Livingstone corer. The primary aim of using such a large diameter core tube was to increase the volume of sediment available for macrofossil analysis from individual sections, thereby increasing the likelihood of detecting infrequent biological remains, whilst leaving sufficient material for other geochemical and biological proxy analyses. Deployment of the large-diameter piston corer in the field required a minimum of four personnel working from a well-anchored platform. The working platform used in this study consisting of two inflatable boats joined by a wooden frame, cores were retrieved from the space between the boats.

Figure 2.12 Core sampling "platform" (core HGBO1)

All cores were kept vertical and once on shore, notes and measurements were made of where distinct layers, colour changes and visible biological remains occurred, such as marl, peat and mollusc rich sections. Once the corer head and piston were

removed, the sediment in the tube was promptly extruded and sliced into 1 cm sections using rods to gradually force the bottom extrusion bung up the core tube. A 1 cm width Perspex ring of the same diameter as the core tube was held over the core top, which acted as the measuring guide before slicing the sediment with a piece of stainless steel sheet. From each 1 cm section, a sub-sample for contaminant analysis was removed from the central area to avoid contamination from the core edge. Approximately 20 cm^3 of wet sediment was placed in a 30 ml glass vial with a foil-lined lid. To aid analyte preservation for subsequent organotin and organic biocide analyses, these sub-samples were stored on ice in the dark, until freezing to -20°C upon returning to the laboratory. The remaining sediment from each section, ~130 cm^3, was placed in labelled, sealable plastic bags and stored in the dark at <4 °C.

2.4.2 Standard Livingston corer

Core HGB02 was collected with a standard 7.4 cm I.D. Livingston corer. It was extruded and sectioned into 2 cm intervals, with the removed sediment samples being placed in labelled, sealable plastic bags and stored in the dark at <4 °C. Through the top 90 cm of core HGB02, the redox potential of the sediment was analysed at 2 cm intervals as each successive slice was removed. An electronic meter was used, with the probe pushed 1 cm into each freshly exposed sediment layer, which aimed to obtain results before significant oxidation of the sediment occurred. Cores HICK1 and BART9 were also taken with a 7 cm I.D. Livingston corer. HICK1 was extruded at 0.5 cm intervals and BART9 at 1 cm intervals. Sample collection and storage followed the aforementioned protocol.

2.5 Laboratory methods - contemporary environmental analysis

2.5.1 Total suspended solids and chlorophyll *a* analyses

TSS sample filters were oven dried over-night at 105 °C and weighed to 4 d.p. on an electronic balance. The samples were then combusted at 550 °C for one hour in a muffle furnace, to remove the organic fraction, after which the filter paper was reweighed. Corrections for mass loss of the filters at high temperature were made with simultaneous triplicate analysis of blank filters. Concentration of total solids, organic and inorganic fractions are presented as mg l^{-1} through calculation of the mass lost after each successive temperature treatment.

Chlorophyll *a* concentration was determined using the cold acetone extraction method (APHA 1992). The filter paper was macerated in a pestle and mortar, with the entire contents rinsed into a glass tube with 90% acetone. This was left overnight, in a refrigerator in the dark, in order to extract the algal pigments into solution. The tube was centrifuged to separate the solids, with the supernatant analysed colourimetrically with a UV/VIS spectrophotometer. An acetone blank was used as the absorbance baseline, with wavelengths measured at 750, 663, 480, 430 and 410 nm for each sample. Chlorophyll *a* concentration was expressed as $\mu g\ l^{-1}$ using the following formula: -

$$\text{Chlorophyll } a = \frac{11 \times (\text{Abs } 633 - \text{Abs } 750) \times v}{Vp}$$

Where v = volume of acetone extractant (ml), V = volume of water filtered (ml), p = pathlength (cm) of the spectrophotometer cell (in this case 1 cm) (Goltermann & Clymo 1971).

2.5.2 Sediment characterisation

2.5.2.1 Loss on ignition

Percentage water content of each core section and surface sediment sample was determined by oven drying a weighed sub-sample in a crucible to constant mass at 105°C. Weight loss was calculated and expressed as a percentage of the original sediment wet weight to give percent water content. Percentage organic matter content was determined using standard loss-on-ignition (LOI) procedures by treating samples in a muffle furnace at 550 °C for 1 hour and calculating the weight loss. Percentage carbonate content was determined through returning samples to the furnace at 950 °C for 1 hour before a final reweighing (Dean 1974). All weights were recorded to 4 d.p. on an electronic balance with the final organic matter and carbonate results expressed as percentages of the sample dry weight.

2.5.2.2 Particle size distribution

Preparation of sediment samples followed a standard methodology (Rowell 1994). This involved gently diaggregating approximately 5 g of air-dried sediment and sieving through a 2 mm mesh. Around 1 g of the fines was weighed to 3 d.p. and

placed in a 50 ml glass conical flask with 5 ml of hydrogen peroxide to remove the organic matter. Another 10 ml of hydrogen peroxide was added and left overnight. The mixture was gently heated on a hotplate with repeated addition of hydrogen peroxide until all effervescent reaction had ceased. The liquid was then boiled off to complete dryness, the cooled flask and contents reweighed, with the remaining inorganic sediment fraction calculated. To disaggregate and disperse the dried inorganic fraction in a solution, 30 ml of ~2% "Calgon" solution was added and left overnight on a wrist-action shaker.

Analysis of the disaggregated sediment was performed on a Beckman Coulter LS™ 13 320 Laser Diffraction Particle Size Analyser with an integrated autosampler. Prior to analysis the samples were sonicated for 10 seconds. The particle size range measured was between 0.4 – 2000 µm. Percentages of clay (0.4 – 2 µm), silt (2 – 64 µm), fine sand (64 – 200 µm) and coarse sand (200 – 2000 µm) fractions were determined for each sample.

2.5.3 Organotin analysis

The gas chromatography methodology for trace environmental analysis and quantification of organotin compounds is well established (Waldock and Waite 1994;Quevauviller *et al.* 1994). The most common sample preparation procedures use an organic solvent to extract organotins from the matrix, followed by derivitization using a hydride or a Grignard reagent (Quevauviller *et al.* 2001).

Organotin analysis on surface and core sediment was performed at The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) facilities at Burnham-on-Crouch, Essex, and Lowestoft, Suffolk. Their standard extraction, detection and quantification methodology was followed (Waldock *et al.* 1989). Samples were extracted wet, and a separate total solid determination was performed to express the result on a dry weight basis (Thomas *et al.* 2000). A tripropyltin internal standard was added to each sample prior to extraction to act as a surrogate compound to correct results for variation in the extraction efficiency from each sample. Organotin compounds were extracted from the sediment matrix by sodium hydroxide and methanol, converted to hydrides and partitioned into hexane. Derivatives were then analysed by gas chromatography with flame photometric detection (GC-FPD). Quality control in each sample batch included an analytical blank; a reference

sediment (BRC-646) spiked with known concentrations of the target organotin compounds; and a Response Factor sample (containing known concentrations all butyltin target compounds and TPT internal standard) which was repeatedly analysed prior to every three environmental samples. Environmental sample concentration was calculated by the peak height of each butyltin compound as a percentage of the peak height of the relevant standard in the previous Response Factor sample. The method limit of detection (LOD) was approximately 2 ng g^{-1} for butyltin species and >50 ng g^{-1} for triphenyltin (Waldock *et al.* 1989). Operationally the Limit of Quantification (LOQ) were usually higher, as measurements from environmental samples were corrected according to the relative peak height of the previous Response Factor sample which corrected for instrumental drift. In this study organotin concentrations are expressed in terms of the cation (TBT^+) as ng g^{-1} of dry sediment. Conversion of organotin concentrations in the literature reported as ng g^{-1} (as Sn), were mulitiplied by a factor of 2.44 and 1.96 for TBT and DBT respectively. Molecular masses of 290.04 g mol^{-1} for TBT^+ and 232.7 g mol^{-1} for DBT^+ were assumed (Selck *et al.* 2002).

2.6 Laboratory methods – palaeolimnological techniques

Table 2.3 gives a summary of the various analytical techniques and methods performed on each sediment core.

Table 2.3 Summary of analyses performed on each sediment core

Core	SALG1	HGBO1	HGBO2	HICK1	BART9
Core depth (cm)	0 - 86	0 - 85	0 - 164	0 - 76	0 - 74
Sediment analysis		*Number of analyses*			
Dry weight, loss-on-ignition, carbonate (%)	86	85	76	*152* [a]	*74*
TBT (ng g^{-1})	17	18		13	18
DBT (ng g^{-1})	17	18		13	18
MBT (ng g^{-1})		18		13	18
^{210}Pb radiometric dating	*8*	*8*		*12*	*10*
Organic carbon (%)	22				
Organic nitrogen (%)	22				
Organic ^{13}C (‰)	22				
Fossil pigments (µg g^{-1})	*20*				
Particle size distribution (% of fractions)	9				
Total phosphorus (mg g^{-1})	24	25			
NaOH-extractable phosphate (mg g^{-1})	24	25			
Redox (mV)			42		
Munsell colour analysis	33	43	10		
Sub-fossil analysis					
Macrofossils	20	17		13	
Cladocera	15	13			

a – numbers in italics represent analyses not performed by the author

2.6.1 Cladoceran sub-fossil remains

Preparation of cladoceran carapaces, head shields, post abdomens and anal claws, for identification and enumeration, followed a method adapted from Korhola and Rautio (2001). Approximately 1 g of wet sediment for analysis was weighed to 4 d.p. The sediment was then heated to around 80 ºC in 100 ml of dilute potassium hydroxide solution to de-flocculate the material and remove organic matter. After an hour, each sediment sample was sieved through stacked 150 µm and 50 µm diameter mesh sizes. Each fraction was carefully washed and then rinsed with deionised water into a pre-weighed plastic pot. A few drops of safranin stain were added to the sample before re-weighing prior to analysis.

Identification and enumeration was performed under a compound microscope. Samples were mixed gently prior to pipette removal of a small portion, which was placed on a non-permanent Lund cell for viewing. Counted material was not returned to the original sample pot. The minimum number method was used to tally

the lowest possible number of individual cladocera that could be present in the sample from the counted remains. This method accounted for variation in the number of different types of remain present from the whole organism, eg. head shields and carapaces. Total counts from the two separately enumerated size fractions were combined. No less than 300 individual cladocera (calculated using the minimum number method) were enumerated from each sample. This method often entailed counting a total of over 500 separate remain types from each sample to achieve a minimum number of greater than 300. Each sample pot was reweighed after counting, enabling determination of the proportion of the total sample analysed, and subsequent quantitative expression of the results. Trichosclereid leaf cells from Nymphaeaceae, either *Nuphar lutea* or *Nymphaea alba*, were also enumerated using this method. Cladoceran chitinous remains and ephippia (in the macrofossil analysis) were identified using standard reference works (Frey 1959;Flößner 1972;Amoros 1984;Margaritora 1985;Alonso 1996).

2.6.2 Macrofossil remains

Macrofossils were separated and visually examined using standard procedures as outlined in (Birks 2001). Around 70 cm^3 of wet sediment was analysed from 1 cm sections taken with the wide diameter Piston corer, cores SALG1 and HGBO1. From HICK1 the volume analysed was between 10 cm^3 and 20 cm^3 depending on the amount of sediment available. Sediment volume was determined through displacement of water in a measuring cylinder with the wet weight of each sediment sample also recorded.

The sampling interval for macrofossil analysis through cores SALG1 and HGBO1 was a 1 cm thick section analysed every 5 cm from the 2.5 cm to 82.5 cm mid-depth samples. The total number of samples analysed was 20 for SALG1 and 17 for HGBO1. Between 32 cm and 42 cm depth in SALG1 an increased frequency of samples were analysed at every 2 cm. This section was where TBT first appeared in the core profile; therefore a finer resolution in the variability of macrofossil remains was sought. The sampling interval through core HICK1 was a 1 cm thick section (composed of pooled 0.5 cm sections) analysed every 2 cm starting at 2 cm through to 22 cm depth.

Prior to macrofossil analysis, the wet sediment sub-sample was soaked for 20 minutes in an equal volume of 10% "Calgon" solution (35.7 g sodium hexametaphosphate & 7.9 g sodium carbonate dissolved in 1 l deionised water) to de-flocculate the organic matter and clay particles. Rinsing through stacked sieves with 300 µm and 125 µm diameter mesh sizes, divided the remains into fractions to facilitate identification and counting. Counting was performed on a glass Petri dish under a light microscope. The larger fraction was counted in its entirety and contained macrophyte seeds, charophyte oospores, leaf fragments, mollusc and other invertebrate remains. The smaller fraction contained, small chironomid head capsules, macrophyte leaf spines and smaller seeds. The smaller fraction was diluted into 200 ml of distilled water and thoroughly mixed, with a 10% volume sub-sample removed (20 ml) by a syringe for counting. Cladoceran epphipia were also enumerated using this method.

In the case of the HICK1 sediment samples, there was a limited volume available for sieving and previous test sieving found the remains to be of a relatively small size. To maximise the total number of remains counted a smaller 250 µm mesh was used to separate the largest fraction, with all remains >250 µm counted. Again 10% volume of the smaller fraction (125-250 µm) was counted. The HICK1 macrofossil data were expressed as a flux, as number of remains per cm^{-2} $year^{-1}$. The radiometric dating of this core was of high resolution and expression of remains as a flux accounted for the dramatic increase in sedimentation rate through the top 10 cm of the core.

From the sediment samples analysed for macrofossils over 90 different plant and animal remain types were categorized. Given the wide range of organisms, the extensive identification knowledge required and often partial preservation, the taxonomic level to which individual remains could be classified varied widely. Where possible, species level identification was achieved for nearly all aquatic angiosperm seeds and vegetative remains; cladoceran ephippia (resting eggs); bryozoan statoblasts (resting stages) and mollusc shells. Where specific identification could not be determined, an aggregate grouping was given. For the Trichopteran (caddis fly), remains, chiefly the cases and fronto-clypeus (head shields); Hirudinea (leech) and triclad (flatworm) egg cocoons; dipteran (midge) larval headshields; amoebal tests; oribatid mites; and charophyte oospores, family level or above was achieved.

The following literature, including specific keys and field guides were used to aid identification of the following remain types; plant seeds (Bertsch 1941;Katz, Katz, and Kipiani 1965); macrophyte vegetative remains (Lansdown 1999); fish scales (Maitland 2004); molluscs (Ellis 1962;Macan 1977;Janus 1982;Kerney 1999); bryozoans (Mundy 1980); general invertebrates (Fitter and Manuel 1986;Olsen, Sunesen, and Pedersen 2001).

2.6.3 Radiometric dating

The radiometric analysis of core sediment samples was performed and interpreted by P.G.Appleby of the Environmental Radioactivity Research Centre, University of Liverpool. Samples were analysed for ^{210}Pb, ^{226}Ra and ^{137}Cs by direct gamma assay using Ortec HPGe GWL series well-type coaxial, low background intrinsic germanium detectors (Appleby *et al.* 1986). ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra by the 295 keV and 352 keV γ-rays emitted by its daughter isotope ^{214}Pb following 3 weeks storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 keV and 59.5 keV respectively. The absolute efficiencies of the detectors were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy γ-rays within the sample (Appleby, Richardson, and Nolan 1992).

2.6.4 Sediment carbon isotope (δ^{13}C) and total nitrogen analysis

22 samples from core SALG1 were analysed by the NERC Isotope Geosciences Laboratory, Keyworth, Nottingham, for δ^{13}C and total nitrogen. Preparation of the organic sediment fraction followed that given by Leng (2003). 1 g of sample was treated with 50 ml of 5% HCl for 12 hours. The samples were washed and dried before finally being ground to a fine powder in an agate pestle and mortar. Analysis was performed by combustion in a Carlo-Elba 1500 on-line to a VG Triple Trap and Optima dual-inlet mass spectrometer. δ^{13}C values were calculated to the VDPB scale using a within-run laboratory standard (BROC1) calibrated against NBS-19 and NBS-22. Replicate analysis of well-mixed samples indicated a precision of $\pm$ <0.1% (1 S.D.). Total organic carbon and nitrogen were also calculated (to give C/N ratios), and these were calibrated against an acetanilide standard. Replicate analysis of

standards indicated a precision of ± 1.0% (1 S.D.) for carbon and ± 0.2% (1 S.D.) for nitrogen.

2.6.5 Sediment total and non-apatite inorganic phosphorus

Following the work of Brenner *et al* (1999b), total phosphorus (TP) and NaOH extracted phosporus (NaOH-P) were determined from sediment samples following the method given in (Ruban *et al.* 1999). The NaOH-P fraction was extracted by shaking 200 mg of dried sediment in 20 ml of 1 mol l^{-1} NaOH solution for 16 hours. 10 ml of the supernatant was then further extracted in 4 ml of 3.5 mol l^{-1} HCl for 16 hours. TP analysis was performed on 200 mg of sediment that had been calcinated at 450 ºC for 3 hours. This material was then extracted in 20 ml of 3.5 mol l^{-1} HCl for 16 hours.

Phosphorus content of extracted samples was determined spectophotometrically by the molybdenum-blue method of (Murphy and Riley 1962). The final supernatant for analysis was diluted so the acid concentration was <0.5 mol l^{-1}, which has been found to be optimal for this reaction (John 1970). Quantification of samples was against an external standard calibration, which was equivalent to a sediment P concentration of 0.2 – 2.7 mg g^{-1}. Linearity over the range gave correlation coefficients >0.99%. RSDs from triplicate analysis of environmental samples were <5%.

2.6.6 Fossil pigment analysis

Dr S. McGowan performed fossil pigment analysis on 20 frozen sediment samples from core SALG1, whilst based at the Limnology Laboratory, University of Regina, Canada,. High Performance Liquid Chromatography (HPLC) analysis was used to quantify characteristic chlorophyll and carotenoid compounds after extraction of the pigments using standard protocols (Leavitt and Hodgson 2001).

2.7 Statistical methods

2.7.1 Contemporary biocide and environmental data

To determine the relationships between variability in the contemporary biocide and environmental variable data, Pearson product moment correlations (*r*) and Student's T-tests (*t*) were used. Normality in the datasets were tested using the Anderson-Darling method, with data accepted as normal if $p > 0.01$. If data were below an acceptable normal distribution criteria, suitable transformations such as log, square root or Box-Cox were employed to meet the statistical assumptions of each test. These and other comparative statistical analyses were performed using Minitab (release 13.42) for Windows.

Where several environmental variables were thought to influence biocide concentration in the contemporary dataset, gradient analyses were performed using Canoco 4.5 for Windows (Ter Braak and Šmilauer 2002). The ordinations were used to summarise the multivariate data and uncover the underlying latent structure of the data. Linear methods were used in all indirect and direct gradient analyses, as the length of the community compostion gradients (in this case biocide concentrations) were all < 4 (Lepš and Šmilauer 2003). This measure was determined using detrended correspondence analysis (DCA).

2.7.2 Palaeolimnological data

Zonation of stratigraphic cladoceran and macrofossil proxy data through each core was achieved through cluster analysis. A variety of constrained clustering techniques were employed using the program ZONE (Juggins 1991). All clustering techniques have inherent limitations and under certain circumstances can provide misleading results. In order to prevent this, several methods were used including constrained single link clustering (CONSLINK) and constrained incremental sum of squares clustering (CONISS). Only results that were common between all analyses were presented as valid clusters in the stratigraphical data. The major divisions between clusters of consecutively analysed samples were identified and labelled in each biostratigraphical diagram (see Appendix 9.8).

An application of gradient analysis is to provide a single value or score for mulitple variables, the so-called dimension reduction technique. Within the biological proxy

data, where numerous species were identified from distinct organism communities, e.g. cladocerans and molluscs, principle component analysis was performed to provide a summary score for the community structure. The axis 1 eigenvalues were then plotted to reveal changes in community structure through the cores.

The Shannon diversity index (*H*) is an index that is commonly used to characterize species diversity in a community, which accounts for both abundance and evenness of the species present (Rosenzweig 1995). This method was used to assess changes in cladoceran species diversity through cores SALG1 and HGBO1. The proportion of species *i* relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

CHAPTER 3 – ORGANIC BIOCIDE ANALYTICAL METHOD DEVELOPMENT

3.1 Introduction

3.1.1 Overview of analytical techniques

The analysis of organic booster biocides from environmental samples is less well established, in terms of methodological standardization and availability of certified reference materials, compared to environmental quantitation of TBT. The analytical techniques commonly employed are also different. Triazine and phenylurea compounds, such as Irgarol 1051 and diuron considered in the present study, are thermolabile at the high temperatures involved in GC separation, therefore less severe analytical conditions are necessary for highly sensitive quantitative analysis (Agilent Technologies 2002). This requirement has led to improvements in the sensitivity, accuracy and sample throughput of High Performance Liquid Chromatography (HPLC) coupled with Mass Spectrometry (MS) methodologies, which are based in an aqueous medium at ambient temperatures (Ferrer and Barcelo 1998;Geerdink *et al.* 1999;van der Heeft *et al.* 2000;Stipicevic *et al.* 2003;Stoob *et al.* 2005). Other benefits of HPLC over GC methodologies include removal of the derivatisation stage and the direct analysis of aqueous samples, which both increase sample throughput (Hernandez, Sancho, and Pozo 2005). The main steps involved in trace level determination of organic biocides using HPLC-MS based analytical systems are outlined.

In order to maximise the amount of target analyte from individual samples available for quantitation, a pre-concentration and clean-up step is required for highly sensitive environmental analysis (Hennion, Cau-Dit-Coumes, and Pichon 1998). Solid phase extraction (SPE) has become the method of choice for combination with HPLC-MS, as the initial extraction of analytes from the aqueous sample is based on the same physio-chemical principals as the LC separation (Hennion 1999). SPE involves passing aqueous sample through a sorbent material onto which analytes from the sample matrix are retained according to the specific hydrophobic properties of each analyte. Complete automation of online SPE procedures have been successfully used in environmental analyses of organic biocides (Slobodnik *et al.* 1997;Patsias, Papadakis, and Papadopoulou-Mourkidou 2002). This has had the effect of increased throughput and enhanced reproducibility, especially for large batches of samples (Hennion 1999).

If molecular weight and structural information are required for unequivocal identification of trace environmental contaminants, analytes entering the MS must be charged. Studies have demonstrated that HPLC-atmospheric pressure chemical ionisation (HPLC-APCI) is a particularly suitable ionisation technique for analysis of neutral organic herbicides (Thurman, Ferrer, and Barcelo 2001;Asperger *et al.* 2001). The APCI interface utilises a gas phase chemical ionisation which is better suited than Electrospray Iosnisation (ESI) for ionisation of medium molecular weight species of moderate polarity (Agilent Technologies 2002), such as the triazine and phenylurea herbicides. This is due to the "soft" ionisation that produces relatively simple spectra (Thomas 1998). As a result of these advances in the instrumentation available, several SPE-HPLC-APCI-MS analytical techniques have been specifically applied to the trace detection of Irgarol and diuron in surface waters (Thomas 1998;Ferrer and Barcelo 1999a;Martinez *et al.* 2000;Almeida Azevedo *et al.* 2000a;Gimeno *et al.* 2001) and sediment (Martinez and Barcelo 2001). Furthermore, MS/MS techniques based on the isolation of a specific analyte ion (the precursor), with subsequent fragmentation then forming characteristic product ions, is a method that allows much lower quantification limits to be achieved, even in the presence of complex matrices. Jeannot *et al* (2000) reported MS/MS sensitivity was up to 10 times greater that simple MS quantification on the precursor ion alone.

The method development process is therefore very important for SPE-HPLC-APCI-MS analyses. The lack of standard methodologies, due to the wide variety of compounds for analysis and instrumentation available, means any such method must have acceptable accuracy, reproducibility, repeatability and validation if robust quantitation is to be acheived. To achieve maximal sensitivity, the separate analytical stages of extraction, chromatographic separation, and ionisation must all be specifically optimised for each target analyte under investigation.

Certain methodological limitations must also be overcome, for example amospheric pressure interfaces, such as APCI, are susceptible to ion suppression effects upon target analytes in the MS, due to interfering substances co-extracted from the sample matrix (Benijts *et al.* 2004). Such matrix effects (ME) can significantly affect overall analytical sensitivity and accuracy by reduction or enhancement of the ionisation efficiency of analytes in the ionisation chamber (Stoob *et al.* 2005;Mezcua *et al.* 2006). Pre-concentration of samples through SPE makes this situation worse,

as the interfering substances are likely to be far more abundant than the target analytes (Gomes *et al.* 2004).

3.1.2 Extraction from sediments

The partitioning behaviour of triazine and phenylurea herbicides in the freshwater environment has led to them being detected in sediments (Gough, Fothergill, and Hendrie 1994;Long *et al.* 1998;Muller *et al.* 2000), as well as in water. Analysis of the concentration of such contaminants requires extraction of the sediment-bound fraction into a medium suitable for the analytical method selected. Techniques used to extract herbicides from sediments for subsequent chromatographic determination, have typically involved liquid-solid extraction (LSE) methods with a suitable organic solvent. Such methods include Soxhlet extraction (Smith 1981), flask-shake extractions (Voulvoulis, Scrimshaw, and Lester 1999a) and ultrasonication (Stipicevic *et al.* 2003). Drawbacks found from using these methods are the length of time required for extractions; degradation of the analytes of interest; problems linked to the evaporation of solvent; and the often large volumes of inflammable and/or toxic organic solvents used, require safe disposal, which can increase overall analytical costs (Andreu and Pico 2004). Increasingly popular instrumental techniques that require less solvent use and produce comparable efficiencies to traditional methods are liquid extraction procedures such as microwave-assisted solvent extraction (MASE) (Camel 2000). To quantify trace concentrations of organic biocides contained in the sediment phase, a suitable extraction methodology, compatible with HPLC-MS, therefore needs to be developed.

3.1.3 Aim

To develop a method for the trace quantitation, at low parts per trillion concentrations, of the antifoulant biocides Irgarol 1051 and diuron in both water and sediment from The Broads, using a fully automated on-line SPE-LC-APCI-MS^n analytical technique. In addition, the agricultural herbicides atrazine, an s-triazine, and isoproturon, a phenylurea, were simultaneously analysed. The agriculturally derived contaminants have similar physico-chemical properties to their antifoulant relatives, and are commonly detected in UK freshwaters (Environment Agency 2000).

3.1.4 Instrumentation and columns

The equipment used throughout the study consisted of an Agilent 1100 Series HPLC and an Agilent LC/MSD Ion Trap (Agilent Technologies Inc., The Netherlands). The HPLC unit included a solvent degasser and quaternary pump; temperature controlled column oven; a temperature controlled Wellplate autosampler, which controlled application of aqueous samples direct to the column; and a UV-Diode Array Detector. Ionisation was performed through an Atmospheric Pressure Chemical Ionisation (APCI) interface. The MSD Ion Trap, a quadrupole ion trap mass spectrometer performed the mass spectrometry. Flow injection analysis (FIA) was performed using a variable flow rate syringe pump (KDScientific, Holliston, MA, USA). The HPLC and MS parameters were controlled via Chemstation and Trap Control (v 4.1) software (Agilent Technologies Inc., The Netherlands) operated on a Hewlett-Packard L1720 personal computer.

A Prospekt 2 Automated Cartridge Exchange (ACE) and High Pressure Dispenser (HPD) (Spark Holland, The Netherlands) performed the automated online SPE, including the cartridge loading and solvent delivery, which were controlled by Sparklink, v 2.3 software. An independently programmable 24 x 10 ml vial Midas autosampler (Spark Holland, The Netherlands) delivered controllable sample volumes in the range 10 - 10000 µl to the Prospekt 2 ACE.

Octadecylsilica columns used in the method development process included a ACE 5 C18, 25 cm x 4.6 mm I.D., 5 µm particle size (Advanced Chromatography Technologies, Aberdeen, Scotland) and a Supelcosil™ ABZ+, 25 cm x 4.6 mm I.D., 5 µm particle size (Supelco, Bellefonte, PA, USA). Disposable guard columns containing the same packing material as the analytical column were used to protect the analytical column from build up of matrix interferences. Guard columns were replaced when system backpressure and/or background interferences increased significantly.

3.1.5 Chemicals and reagents

All standards were of high purity, with Irgarol 1051 (>98% purity) purchased from Sigma-Aldrich (Poole, UK); diuron (99.4%) from Riedel-de-Haen (Seelze-Hanover, Germany); atrazine (>99.9%) from BDH (Lutterworth, UK); isoproturon (>98%) from

Supelco (Bellefonte, PA, USA); and deuterated atrazine-d^5 (97.5%) from Dr Ehrenstorfer (Augsburg, Germany). HPLC grade acetonitrile and methanol (VWR, Poole, UK) and formic acid (50%) (Fluka, Switzerland) were used throughout. All water used in HPLC eluents and standard solutions was delivered from an Elgastat UHQ unit (Elga Labwater, High Wycombe, UK) with quality >18 MΩ cm^{-1}.

Stock standards of target analytes were prepared in acetonitrile to make approximately 50 mg l^{-1} (w/v) solutions and were stored in glass bottles in the dark at -20 °C. From the stock standards, working standards were prepared in HPLC grade water of 2 mg l^{-1} concentration, which were stored in the dark at <4 °C. The working standards were used to make up fresh standard solutions containing target analytes for method development, calibration and QC work. Good Laboratory Practice was followed regarding the amounts of biocide used, solvent loss through evaporation, final concentrations of the stock standard solutions and all Health & Safety protocols for handling toxic substances. All analysed standards had a final acetonitrile concentration of <0.1%.

3.2 Methods

3.2.1 Sample preparation

Prior to use, all glassware was soaked overnight in a 5% solution of Decon 90 detergent, rinsed with de-ionised water, solvent rinsed with acetonitrile and allowed to air dry. Prior to analysis, water samples were vacuum filtered through Whatman (0.45 µm) cellulose nitrate membrane filters, with the filtrate added to 10 ml Midas autosampler vials.

3.2.2 Optimization of SPE and LC separation

A standard mixture containing each of the four target analytes (10 µg l^{-1}) was directly injected into the different LC columns. A variety of different eluents, solvent strengths and flow rates were tested to determine the LC conditions which gave the optimal resolution of analyte chromatographic peaks, whilst maintaining good peak shape. Analyte detection at this stage was by UV-DAD, using previously optimised UV wavelengths of 254 and 240 nm to detect the triazine and phenylurea compounds respectively. Individual analyte retention times and the spectral

characteristics were established by direct injection of single analyte standards. All LC columns were maintained at a constant 40 °C.

Several different SPE cartridge packing materials were tested to demonstrate which type gave the greatest recovery of target analytes. 10 ml of a standard mixture of the four target biocides (1 ug l^{-1} concentration of each) was passed through the SPE cartridges prior to elution to the HPLC-MS with a 40:60 acetonitrile:water mixture at a 1 ml min^{-1} flow rate. Hysphere (Spark Holland, The Netherlands) 10 x 2 mm I.D. cartridges containing C-8 (end-capped), C-18 (end-capped) and C-18 (High Density) sorbents were tested.

Prior to sample application, SPE cartridges were preconditioned with 2 ml acetonitrile to aid sorbent solvation, then equilibrated with 14 ml of HPLC grade water, both delivered at 5 ml min^{-1}. Sample application was delivered at 4 ml min^{-1}, with samples aspirated from the Midas autosampler. Cartridges were then washed with 1 ml of HPLC grade water at 4 ml min^{-1}, followed by a backwash of 1 ml water at 2 ml min^{-1}. Finally, the retained analytes were eluted from the SPE cartridges by automatic valve switching which meant LC eluent was then passed through each cartridge before arriving at the LC column.

3.2.3 Optimization of APCI-MS parameters - ionization, fragmentation and detection

Flow Injection Analysis (FIA) was used to deliver standard solutions (10 µg l^{-1}) to the APCI-MS using a syringe pump (300 µl h^{-1}), into a continuous flow of eluent solution (40:60 acetonitrile-water), via a T-connector. The resultant FIA mixture aimed to match the eluent solvent strength delivered to the MS under analytical conditions. Comparison of the abundance of ions formed (signal intensity) was also evaluated to determine whether a negative or positive ionisation mode gave the greatest sensitivity for each analyte. The quadrupole ion trap mass spectrometer allowed retention and subsequent fragmentation of the quasimolecular precursor ions, giving structural information for each analyte and thus unequivocal identification. Multiple Reaction Monitoring was used for the isolation of each protonated precursor molecule in the Trap, and its subsequent fragmentation to generate characteristic product ions, with the most abundant product ion for each analyte being selected for quantitation (the quantitation ion). The mass charge (m/z) ratio and relative intensity

of product ions in each analytes spectra were compared to published work to ensure correct identification. The fragmentation parameters in the Trap software were then optimised for each analyte to produce the greatest acheivable intensity of quantitation ion formation.

3.3 Results

3.3.1 SPE sorbent and LC conditions

The C-8 and C-18 (high density) SPE cartridges gave acceptable recoveries for the triazine compounds, but not for the phenylureas. C-18 (end-capped) did not give the greatest recoveries for the triazines, but produced more equitable results between the triazines and phenylureas. Therefore Hysphere C-18 (EC) cartridges were used throughout this study. Acetonitrile as the eluent organic solvent produced the greatest recovery for the triazine compounds from the SPE compared to methanol, whereas for the phenylureas, similar recoveries were obtained using either acetonitrile or methanol. Acetonitrile was thus selected as the organic solvent used in the eluent for the remainder of the study.

The first LC column used in an attempt to separate the selected biocides was an ACE 5 C18, 25 cm x 4.6 mm (Advanced Chromatography Technologies). On this column however, the chromatographic peaks for isoproturon and diuron were poorly resolved. Co-elution of these two compounds has been previously observed on certain C-18 LC columns (Gennaro *et al.* 1995). Decreasing the proportion of solvent (acetonitrile) in the eluent increased all analyte retention times, thus increasing separation, but peak shape of the phenylureas became low and wide, which adversely affected sensitivity.

Another C-18 column of similar dimensions, a Supelcosil ABZ+ (Supelco) was used and tested, and was found to effectively separate diuron and isoproturon under the same elution conditions. This column was then used for all subsequent analyses. The optimal eluent solvent strength and delivery rate was found to be an isocratic solution of 35:65 acetonitrile-water at a flow rate of 1 ml min^{-1}.

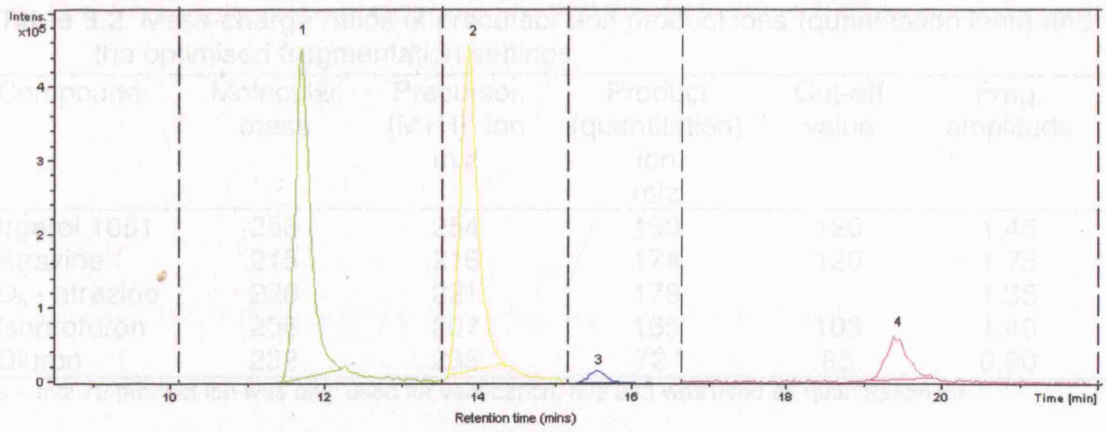

Figure 3.1 MS compound chromatogram (1000 ng l^{-1} standard) showing elution order with the Supelco ABZ+ column and the MS Trap segments (dotted lines) used for each analyte's determination.
1 - Irgarol, 2 - atrazine, 3 - isoproturon, 4 – diuron.

3.3.2 APCI settings and MS detection

The greatest sensitivities for all the analytes investigated were found when positive ionisation mode was used, in agreement with work by (Thurman *et al.* 2001). Table 3.1 gives the optimised APCI and MS acquisition parameters, which were obtained by using the optimisation functionality within the LC/MSD Trap software. Signal intensity and chromatographic peak shape were enhanced with the addition of 0.1% formic acid to the eluent solutions. Addition of the weak acid to the eluent aided analyte ionisation through supplying an extra source of protons (Gao, Zhang, and Karnes 2005). For each analyte, the dominant product ion; m/z ratios of precursor and product; optimal cut-off values; and optimal fragmentation amplitude, are listed in Table 3.2.

Table 3.1 Optimised APCI-MS acquisition parameters for the target analytes.

APCI settings		MS settings	
Ion Source	APCI	Capillary Exit	- 3500 V
Ion polarity	Positive	Corona	4000 nA
APCI Vaporiser Temp	400℃	Skimmer	40 V
Nebulizer pressure	50 psi	Capillary Exit	125 V
Dry Gas flow rate	5 l/min	Octopole 1 DC	10
Dry Gas Temp	325℃	Octopole 2 DC	1.6
		Trap Drive	40
		Octopole RF Amplitude	100 Vpp
		Lens 1	-5
		Lens 2	-60

Table 3.2 Mass-charge ratios of precursor and product ions (quantitation ions) and the optimised fragmentation settings.

Compound	Molecular mass	Precursor $[M+H]^+$ ion m/z	Product (quantitation) ion m/z	Cut-off value	Frag. amplitude
Irgarol 1051	253	254	198	120	1.45
Atrazine	215	216	174	120	1.75
D_5 - atrazine	220	221	179		1.35
Isoproturon	206	207	165	103	1.40
Diuron	232	233	72 [a]	65	0.90

a – m/z 72 product ion was only used for verification, m/z 233 was used as quantitation ion

Figures 3.2 to 3.6 show the optimised fragmentation mass spectra derived from each precursor ion (blue diamond marks the precursor m/z ratio).

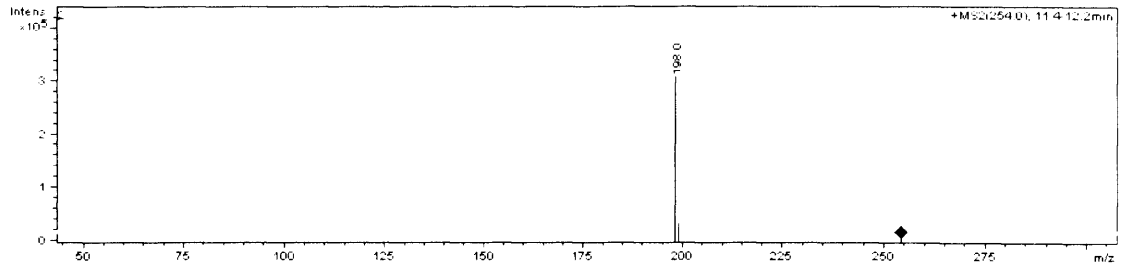

Figure 3.2 Mass spectrum of the fragmented Irgarol $[M+H]^+$ ion (254 m/z)

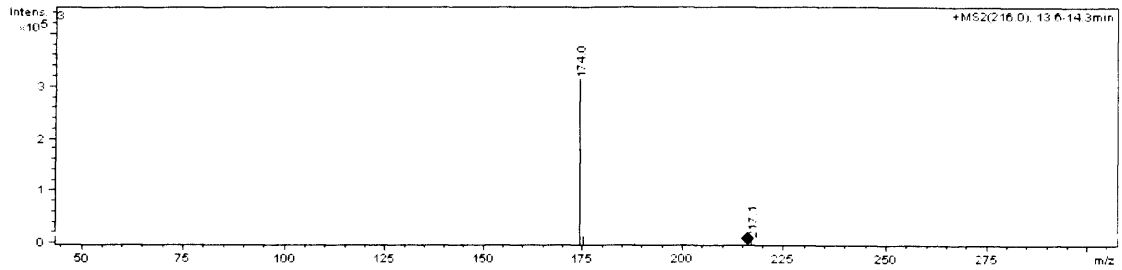

Figure 3.3 Mass spectrum of the fragmented atrazine $[M+H]^+$ ion (216 m/z)

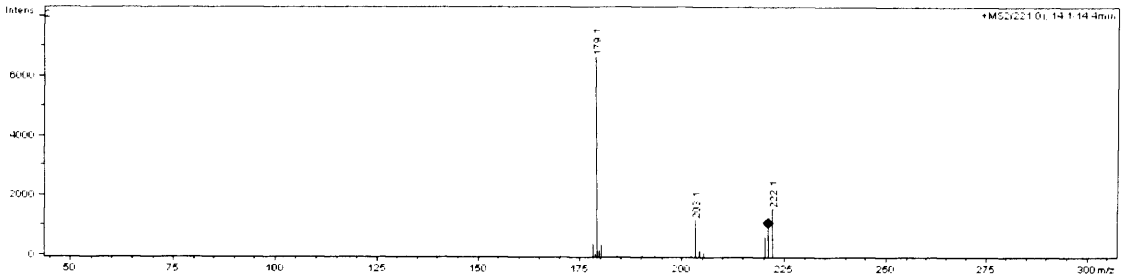

Figure 3.4 Mass spectrum of the fragmented D5 - atrazine $[M+H]^+$ ion (221 m/z)

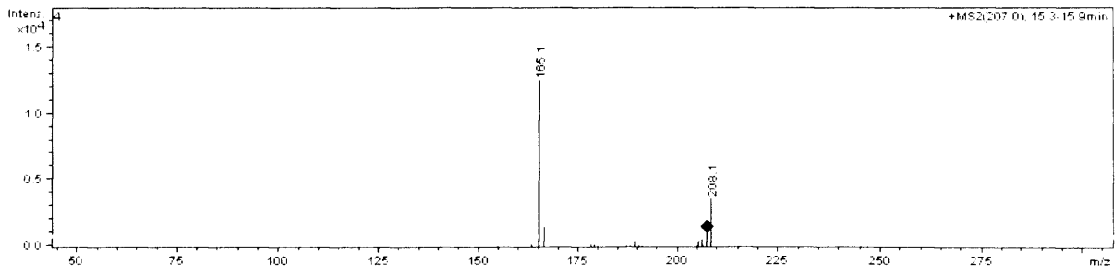

Figure 3.5 Mass spectrum of the fragmented isoproturon $[M+H]^+$ ion (207 m/z)

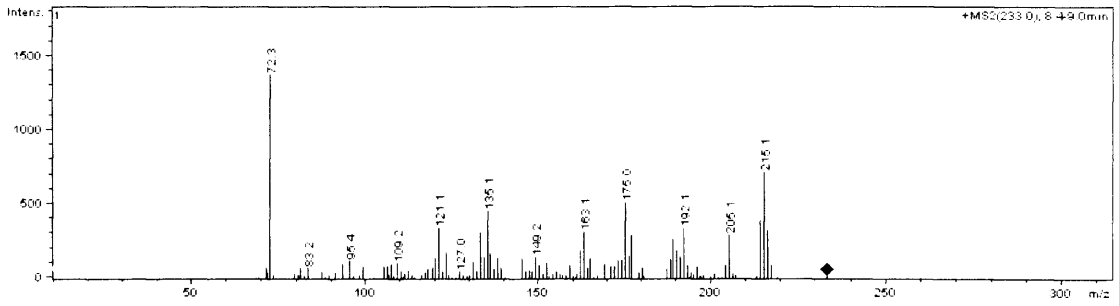

Figure 3.6 Mass spectrum of the fragmented diuron $[M+H]^+$ ion (233 m/z)

Problems arose with the fragmentation of diuron, as Ion Trap MS can only effectively fragment precursor ions to 1/3rd of their m/z ratio (Agilent Technologies 2002). For diuron $[M+H]^+$ (m/z 233), the most abundant product ion formed in positive ionisation mode was 72 m/z, as found in other studies (Steen and et al 1999;Ferrer and Barcelo 1999b;Gimeno *et al.* 2001). This was below the 1/3rd stability threshold of Ion Trap MS. Linearity across the calibration range was thus poor from the fragmented 72 m/z product ion (r = <95%), with unacceptable residual standard deviations (RSDs) of repeatedly analysed standard concentrations (RSDs >20). Quantification of diuron was therefore based on isolation of the 233 m/z $[M+H]^+$ product ion alone (Figure 3.7), which gave acceptable correlation coefficients >0.99 and RSDs < 10.

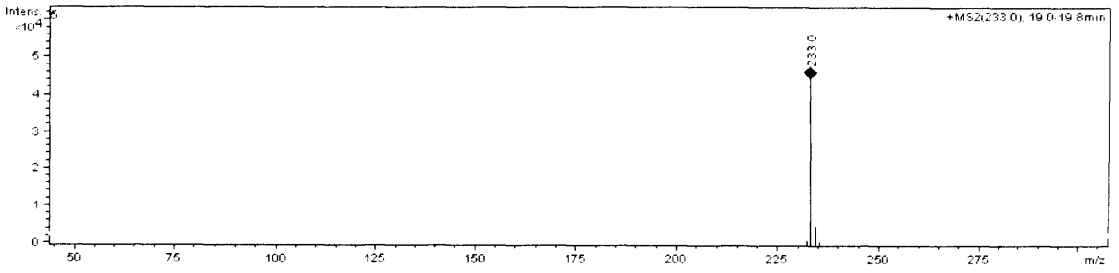

Figure 3.7 Mass spectrum of the unfragmented diuron $[M+H]^+$ ion (233 m/z)

Quantification on the isolated 233 m/z ion reduced sensitivity for diuron, as only chromatographic peaks with signal-to-noise (S/N) ratios >10 (the ratio between peak intensity and background noise) were accepted due to the loss of selectivity. Environmental samples that gave positive results for diuron using this method (ie. 233 m/z chromatographic peaks from environmental samples, at the same retention time (RT) as from the standard solution) were re-analysed with the 233 m/z ion peak fragmented (Figure 3.6). This was to ensure that false positive results were avoided and that the characteristic 72 m/z ion was produced from such peaks. All other analytes were quantified by peak area of quantitation ions with S/N >3 (section 3.3.3.2).

3.3.3 Quality control (QC) and method validation

3.3.3.1 Calibration

An external six-point calibration curve (concentration range 2 – 1000 ng l^{-1}) was utilised for quantification of environmental samples. Proportions of a 1000 ng l^{-1} standard were delivered from the autosampler, as this gave the most accurate and reproducible dilution series. Linearity was achieved for all herbicides over the concentration range, both in standards prepared with HPLC grade water and with cellulose nitrate membrane filtered (Whatman 0.2 µm) River Bure water collected upstream of the navigational limit (Figure 3.8).

Correlation coefficients were all >0.99, indicating acceptable linearity over the concentration range of interest and good performance of the chromatographic method. There was good agreement in the slope and fit for all herbicides between the two matrices tested, indicating the suitability of calibration standards made up in HPLC grade water for comparison with environmental samples. The slope of the curve for diuron in filtered river water had a slightly lower gradient than for HPLC grade water. Ion suppression and/or matrix interferences from the river water may have reduced sensitivity at higher concentrations, but as these data are not replicated, the significance is unknown.

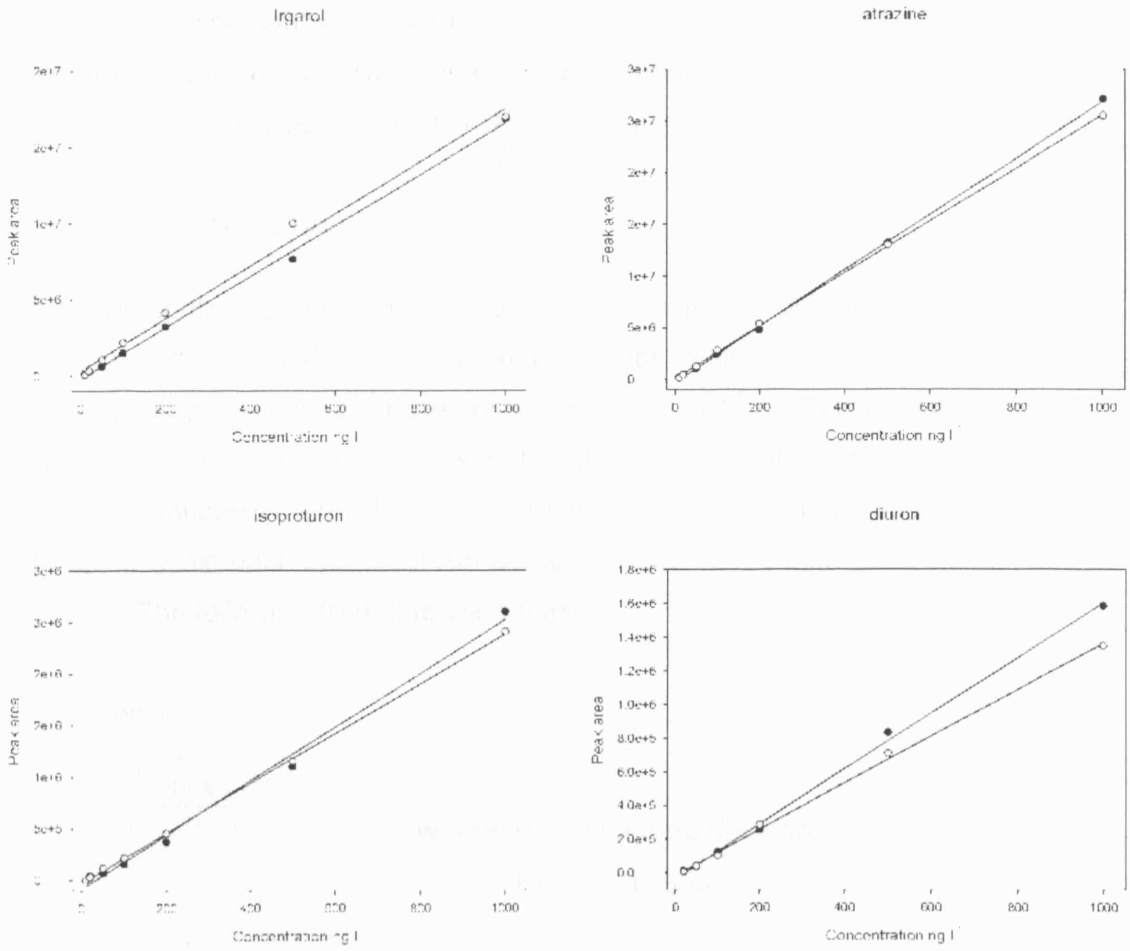

Figure 3.8 Calibration curves of target analytes in HPLC grade water (solid circles) and filtered river water (open circles). R^2 = >0.99 in all cases.

3.3.3.2 LOD

Quantification was based on the chromatographic peak area of characteristic product ions (quantitation ions) or precursor ion only for diuron (Table 3.2). Integration of the peak areas was performed via the QuantAnalysis software (Agilent Technologies Ltd, The Netherlands), with manual integration used on the occasions when the algorithm failed to correctly integrate sub-optimal peak shapes. The limit of quantification (LOQ) was defined as the lowest calibration standard that gave an acceptable S/N ratio within each run. All peaks with S/N ratios >3 were accepted for quantification (Ferrer and Barcelo 1999b), except for diuron where only peaks with S/N >10 were accepted. In comparison to the other target analytes, the reduced sensitivity and selectivity for the determination of diuron gave a higher LOQ of 20 - 50 ng l^{-1}. Sensitivity was greatest for Irgarol, with a consistent LOQ of 2 ng l^{-1}. Atrazine also had consistent LOQ of 10 ng l^{-1} and isoproturon LOQ ranged from 50

to 10 ng l^{-1}. The relative instability between analytical runs for the LOD of the phenylurea compounds was due to the weaker signal intensity compared to that of the triazines produced using this method (Figure 3.1).

3.3.3.3 Precision - repeatability

A quality control (QC) standard (100 ng l^{-1} concentration for each analyte) was analysed after every five environmental samples to check the method repeatability within each analytical run. Potential variation in signal intensity may have arisen through effects from interferring substances in the sample matrices and drift in instrumental performance between samples. Variation in analyte response from QC samples within each analytical run was expressed as residual standard deviation (RSD). The following formulae were used.

Equation 1.

$$\sigma_{n-1} = \sqrt{\frac{\Sigma (PA - \overline{PA})^2}{n - 1}}$$

where σ = standard deviation
PA = peak area

Equation 2.

$$RSD = \frac{\sigma_{n-1}}{\overline{PA}} \times 100$$

RSD's were consistently < 10 %, but in one run the diuron QC samples gave a RSD of 18%. As there there are no certified reference materials for these herbicides the use of % recovery data from spiked samples was used to evaluate the method acceptability.

3.3.3.4 Matrix effects

For the water samples analysed from the wider spatial survey in August 2004, a stable isotopically labelled internal standard (IS) was used to determine the relative level of matrix effects (MEs) within each sample compared to standard solutions made up in HPLC grade water. Deuterated, d^5-atrazine was added to each water sample to give a final IS concentration of 100 ng l^{-1}. Percentage RSD of the water

sample D^5-atrazine peak areas was 5.2 %. As variation in the internal standard signals was relatively low and showed minimal variance compared to the results from HPLC standards, no ME correction was applied to the externally calibrated environmental water concentrations.

3.4 Sediment-bound herbicide extraction

3.4.1 Introduction to microwave-assisted solvent extraction (MASE)

Use of microwave energy to aid organic compound extraction from solid matrices was first achieved in the late 1980s using household appliances (Ganzler, Salgó, and Valkó 1986). Since then specifically designed laboratory microwave systems have emerged with numerous methods having been developed for polar herbicide extraction from sediments and soils (Steinheimer 1993;Molins *et al.* 2000;Papadakis and Papadopoulou-Mourkidou 2002;Shen and Lee 2003). The extraction method usually involves heating a liquid solvent in contact with the sample matrix by microwave radiation. The resultant increase in pressure (in closed vessel systems) and temperature, causes rapid desorption of compounds from the solid matrix and thus diffusion to the solvent (Hoogerbrugge, Molins, and Baumann 1997). Microwave energy causes molecular motion by ionic conductance and dipole rotations. Heat is released as molecules with dipoles (either permanent or induced) align and return to disorder within the electric field generated in the solvent and sample. Rapid heating occurs as the entire sample is irradiated simultaneously (Camel 2000), but the degradative effects on target analytes of high temperatures and lengthy extractions can be avoided (Ganzler *et al.* 1986). A further beneficial effect of extraction by microwave energy is the reported destruction of the matrix macrostructure (Donard *et al.* 1995). Heating of clay, oxides and water in sediments should produce gas bubbles which breakdown the solid particles, therefore increasing the surface area of the solid sample for extraction. These factors make MASE an attractive option for the extraction of triazine and phenylurea compounds from sediments, however the critical extraction parameters require careful consideration.

Solvent choice in the glass reaction vessels is an important parameter that requires optimisation for efficient extraction. The absorption of microwave energy, and thus thermal energy released, is proportional to the dielectric constant of a solvent. In

practice the energy absorbed is also proportional to the solvent polarity (Camel 2000). Water alone has been successfully used in MASE procedures for the extraction of triazine compounds from soils (Xiong *et al.* 1999) and Irgarol from marine sediments (Gatidou, Zhou, and Thomaidis 2004). Water is a polar solvent, therefore it efficiently absorbs microwave energy; readily dissolves triazines; and is an environmentally friendly option, as less organic solvent is used during the course of an analytical run. MASE does not however separate the target analytes from other extracted matrix interferences (Font *et al.* 1998) and therefore some kind of clean up and preconcentration step is required. This can be achieved by SPE methods, which are highly suited for aqueous solutions when coupled with HPLC separation (Papadakis and Papadopoulou-Mourkidou 2002). SPE thus helps to improve the selectivity and sensitivity of the analytical method.

Previous experimental work has shown that extraction temperature, sediment-water ratio, solvent strength and matrix water content are critical extraction parameters that require careful consideration (Molins *et al.* 1996;Hoogerbrugge *et al.* 1997;Molins *et al.* 1997;Patsias *et al.* 2002). In the present study, an optimised MASE method for the extraction of the triazine compounds Irgarol and atrazine from freshwater surface sediment has been developed. Validation of the extraction method and subsequent quantitation of the extracts was tested through analysis of environmental samples collected from the River Bure waterway in August 2004. Problems of target analyte quantitation from sediment extracts, due to co-eluting interferences derived from the sediment matrix itself, are discussed.

3.4.2 Methods

3.4.2.1 Instrumentation

A 1200W MARS-X microwave system (CEM Corp. Matthews, NC, USA) was used throughout, which contained a maximum of 14 closed glass extraction vessels each with a PTFE lid. Temperature and pressure during extractions were monitored via a probe in one of the vessels.

3.4.2.2 Sediment spiking procedure

In order to determine the optimal MASE conditions for the extraction of triazine compounds from riverine sediments, a natural sediment known to be free from

antifoulant biocides through previous analysis (collected from the River Frome, Dorset), was spiked with Irgarol and atrazine to a known concentration. Given the lower solubility and higher K_{ow} values for the triazines (Appendix 9.1), extraction was specifically optimized for these compounds as they were more likely to be found to be associated with the sediments of the River Bure than the phenylurea biocides. The sediment was left to air dry to constant weight, ground with a pestle and mortar, and seived through a 2 mm mesh (Thomas *et al.* 2000). 20 g of the fines were added to a glass jar and spiked with target herbicides to a concentration of 2.5 ng g^{-1} by addition of 2.5 ml of a 20 µg l^{-1} analyte solution. Excess acetonitrile (40 ml) was also added to aid homogenous distribution of analytes within the sediment (Shen and Lee 2003). The slurry was shaken for 1 hour at 300 rpm on a flat bed shaker, followed by 1 hour on a wrist action shaker at 700 oscillations min^{-1}. The acetonitrile was left to evaporate overnight in a fume cupboard, with the final spiked sediment then refrigerated at 4°C for 36 hours prior to extraction. This period of 36 hours was used to ensure that sorption to sediment had reached equilibrium and therefore was better representative of the type of sorption encountered in environmental samples where "slow sorption" effects would likely to have taken place.

3.4.2.3 MASE conditions

All MASE was performed at 80% microwave power. A total extraction time of 3 min and a 1% concentration of methanol in the water extractant were found to be optimal for the extraction of triazines under MASE conditions (Shen and Lee 2003), and were used throughout the study. Sample heating involved a 30 second ramp to the desired temperature, which was held for 2.5 mins, and then allowed to cool to room temperature before opening the vessels. Leaving the vessels to cool prior to opening reduced any loss of vaporised analyte and solvent present in the headspace. The extract was decanted into 60 ml polypropylene tubes, centrifuged at 4000 rpm for 4 mins and the supernatant filtered through Whatman 42 filter papers. 11 ml of filtered extract was pipetted into pre-cleaned Midas autosampler glass vials, with addition of 550 µl of internal standard (2 µg l^{-1} d_5-atrazine solution).

QC standards and analytical blanks were treated as environmental samples, being subject to the same extraction and analytical proceedures. Quantification of target analytes was performed using the SPE-HPLC-APCI-MS^n method detailed in sections 3.2 and 3.3.

3.4.2.4 Optimisation of MASE parameters

Air-drying sediments prior to extraction controlled the water content in the sediment samples, thus reducing the potential effects that water content has been observed to have on extraction efficiency from wet sediments (Onsuka and Terry 1993;Lopez-Avilla, Young, and Beckert 1994). The following steps were performed to determine the optimal sediment-water ratio and temperature for extraction :-

1) To determine the optimum sediment-water ratio for extraction of Irgarol and atrazine, a simple elutriate test was performed on the spiked sediment. 1 g of spiked sediment was extracted into triplicate batches of 15, 20 and 25 ml HPLC grade water. 25 ml represented the largest volume the MASE "GreenChem" extraction vessels could safely hold. Elutriate extractions were performed in 60 ml polypropylene tubes placed in a wrist action shaker for 2 h. Extraction efficiency was calculated through comparison to the signal intensity of a standard at the concentration which respresented the theoretical 100% recovery of all analyte from the spiked sediment. This standard was treated the same as for the spiked sediment, so any analyte losses to the apparatus were accounted for.

2) To determine the optimum extraction temperature during the MASE between 60 – 100 ºC, 1 g of spiked sediment was placed in pre-cleaned glass "GreenChem" closed-extraction vessels (CEM Corp. Matthews, NC, USA) and suspended into the optimal volume of HPLC grade water, as determined in the previously described step.

3.4.3 Results

3.4.3.1 Optimal extractant volume and MASE temperature

For Irgarol the optimal volume of water used for extraction was 20 ml, with 25 ml having a very slightly lower efficiency. For atrazine there was a gradual increase in efficiency with each 5 ml addition of HPLC grade water, with 25 ml having the greatest efficiency (Figure 3.9). Given the greatest extraction efficiency for atrazine at 25 ml, and Irgarol efficiency being very similar between 20 and 25 ml, the larger volume of 25 ml was selected for use in all further method development work and the extraction of environmental samples.

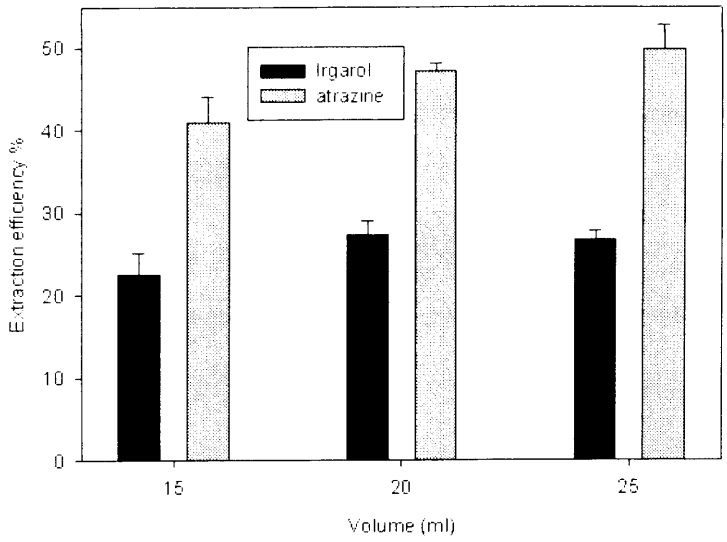

Figure 3.9 Extraction efficiency of Irgarol and atrazine from different volumes of HPLC grade water as extractant.

MASE temperature was optimised for Irgarol and atrazine, with triplicate analysis of spiked sediment extracted at 60, 70, 80, 90, 100 and 110 °C. The results are presented in Figure 3.10 as a percentage of the theoretical 100% recovery from the spiked sediment. Optimal recovery from the spiked sediment for Irgarol was between 80 – 100 °C and for atrazine was at 100 °C. Extraction efficiencies decreased rapidly for both compounds at 110 °C.

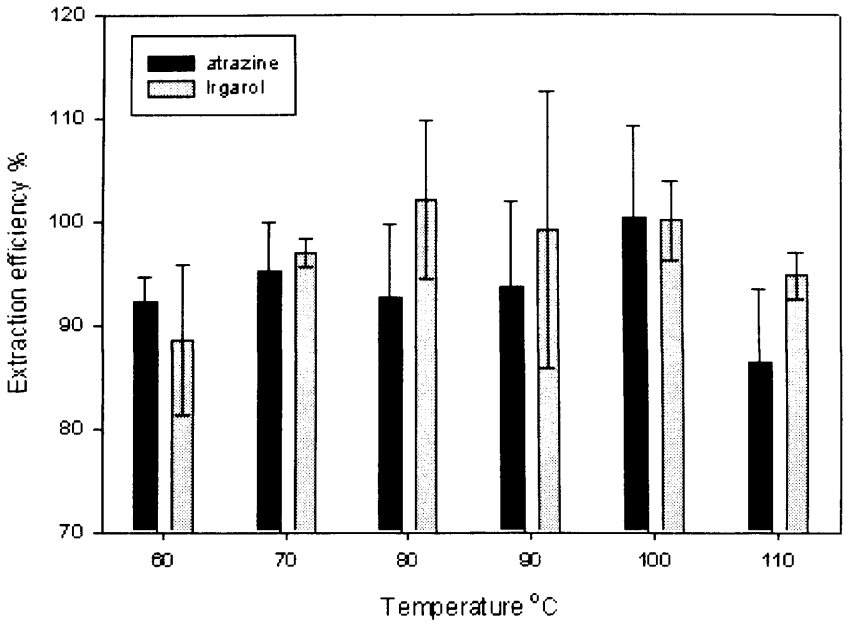

Figure 3.10 MASE recovery efficiencies of triazines extracted at different temperatures

Repeatability of the extraction method was good for both analytes. RSDs of analysed extracts were < 10 (n=3) for all extraction temperatures, except for Irgarol extracted at 90 °C which had a RSD of 13%, which is still below the guideline 30% RSD for extraction methodologies (USEPA 1999). Given the good recoveries for both compounds at 100 °C, a slightly lower temperature of 95 °C was selected for the extraction of environmental samples, as both a compromise to account for Irgarol being equally well extracted at 80 °C and to avoid the negative effects on extraction efficiency observed above 100 °C.

3.4.3.2 MASE method validation

Herbicide extracts obtained from environmental sediment samples were quantified against a 6-point external calibration standard made up in HPLC grade water. As both the external calibration standards and aqueous sample extracts were pre-concentrated using C-18 SPE cartridges prior to LC-MS^n analysis, the analytical sensitivity was found to be similar to that obtained for environmental water samples, i.e. in the low part per trillion range (ng l^{-1}). After calculations to convert the aqueous sediment extract results to sediment associated biocide concentrations, the LOD was 0.1 ng g^{-1} for Irgarol and 0.3 ng g^{-1} for atrazine and isoproturon. Quantitation of the sediment associated biocides from environmental samples was therefore in the low parts per billion range.

The concentrations derived for environmental sediment extracts were corrected for matrix effects. Internal standard (d_5-atrazine) results gave information on matrix effects from co-extracted interfering compounds, as expected to arise from extraction from such complex matrices. RSDs for IS from sediment extracts was high with an average of 28.5%, compared to 5.2% from the environmental water samples (see section 3.3.3.4). Sediment-associated biocide concentrations were corrected according to each samples IS response, as a proportion of the following QC standards (matrix free) IS response. This method gave a percentage difference for each sample result, compared to the QC standard, which was then used to correct the sample concentration value.

Variability in target analyte percentage RSD values for the QC samples during the sediment analysis was slightly higher relative to those obtained using the same analytical method for the August 2004 water samples (Table 3.3). The repeatability

of the sediment MASE method in the present study was as good as a similar triazine MASE method (Gatidou *et al.* 2004).

Table 3.3 Percentage RSD for QC samples from August 2004 sediment and water analyses

	Percentage RSD	
	Irgarol	Atrazine
Sediment samples	12.4	10.1
Water samples	1.5	5.6

This observation suggests that despite the sediment extraction QC samples being prepared to be free of matrix interferences, they may have become contaminated during the extraction proceedure; there was carry-over in the LC column of interferences extracted from the sediment matrices; or a possible build-up of residues in the APCI chamber occurred. All of these sources could have caused the observed greater variability in the sediment QC sample results. However, analytical blanks were run and were all found to be free of target analytes, so contamination from the extraction glassware is unlikely. The QC methodology adopted however cannot fully rule out the occurrence of the latter two sources of interference to the QC samples in the sediment analysis. Incorporation of analytical blanks after environmental samples and at the end of each run may help identify the presence of any potential interfering compounds, especially if analysed in full scan mode. The IS results from the sediment extracts also provide some information on the matrix effects experienced.

The only measured sediment characteristic that had a significant correlation with the internal standard response was sediment organic matter content ($r = -0.552$; $p = <0.001$, Figure 3.11). No relationship between d5-atrazine and atrazine signal intensity was observed, indicating no measurable negative effects of the close elution of these two compounds. The MRM functionality and high selectivity of the MS^n Ion Trap helps to avoid such reductions in signal intensity between target analytes with similar retention times and m/z ratios.

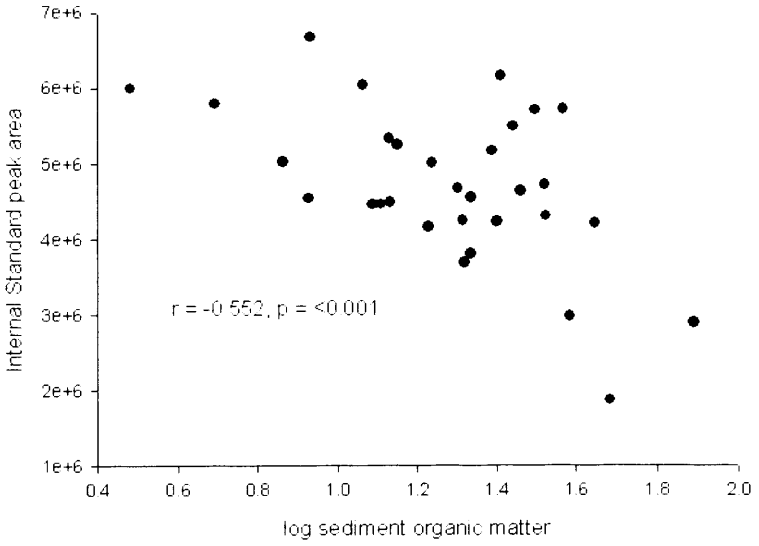

Figure 3.11 Relationship between D^5-atrazine response and sediment organic matter content

Sediments with a higher organic matter content resulted in extracts that gave on average, lower IS responses (right-hand side of Figure 3.11). Greatest ion suppression in the APCI appears to have occurred in sediments with a higher organic matter (OM) content. The most probable process influencing this observation is co-eluting interferring substances successfully competing for ionisation in the APCI and subsequently reducing the analyte signal intensity. The IS variation between sediment samples with similar percent OM content (central data cloud in Figure 3.11) may be possibly explained by variation in the type and physico-chemical properties of humic, or other common matrix interferences, present at different sample sites. However identification of these specific compounds and their relative influence on analyte signal is beyond the scope of this study.

3.5 Discussion

The determination and quantification of ultra-trace levels of a suit of organic biocides from environmental matrices, as outlined in the present chapter, represents an optimised analytical method using a specific SPE-LC-MS set up. Improvements in the method development procedure would have enabled a greater degree of statistical justification for the final setting of various analytical variables at the SPE and LC stages. For example, replicated experiments testing the recovery efficiency of different solid phase sorbents in the SPE cartridges would have increased the

confidence that optimal retention of target analytes had been achieved. Similarly, the final selection of eluent variables such as flow rate and solvent type and strength could have been more robustly justified, had a more systematic approach to optimisation been adopted. Following an optimization matrix or orthogonal array design when experimentally determining the performance of several variables simultaneously would have provided such results. The SPE-LC-MS method adopted however performs well in terms of repeatability, reproducibility, specificity and sensitivity.

Greater sensitivity in analysis of the phenylurea compounds could be achieved with further optimisation of the ionisation mode of the APCI (positive or negative) in combination with variation in solvent type and strength. Use of gradients in eluent solvent strength during elution from the LC was not explored, but could possibly be used to enhance the signal from the analytes with the longer retention time, such as the phenylureas.

3.6 Conclusions

The MASE method developed has been shown to be a reliable, fast and efficient proceedure for the extraction of Irgarol and atrazine from freshwater sediments. When combined with the highly sensitive and selective SPE-LC-APCI-MS^n method developed in the present study, analysis of the aqueous MASE extracts has produced sediment-associated biocide concentration results in the low parts per billion (ng g^{-1}) range. Matrix effects within sediment samples were identified and corrected for, ensuring that biocide quantitation was as accurate as possible. The method is therefore highly suitable for determination of the spatial and temporal contamination patterns of triazine biocides in the environment.

Matrix interferences appeared to be carried over between analysed samples, as the RSDs of QC standards in the sediment analysis were slightly higher than expected. In order to decrease this potential source of error within the method, greater flushing of the analytical column after each sample application would help reduce any build up of interferences within the instumentation and clear the LC column between samples. Also running QC standards more frequently, for example after every three environmental samples, rather than every five, would give greater confidence in identifying and quantifying sources of variation in IS and analyte signal intensities.

CHAPTER 4 – CONTAMINATION OF ORGANIC ANTIFOUL BIOCIDES ALONG THE RIVER BURE WATERWAY

4.1 Introduction

Organic antifoul paint biocides have been reported to accumulate to detectable levels in a variety of natural waters and sediments around the globe (Konstantinou and Albanis 2004). Highest concentrations are associated with areas of dense boat mooring (Gough *et al.* 1994;Bowman *et al.* 2003) and where containing paint flakes enter the aquatic environment (Thomas *et al.* 2003). Strong seasonality of concentrations has also been shown in popular boating areas (Voulvoulis *et al.* 2000;Haglund *et al.* 2001). Of the organic biocides used in antifoul paint formulations, only Irgarol 1051 has been used solely as an antifoulant. Determination of environmental contamination of this compound can therefore only be ascribed to that leached from antifoulant painted boat hulls. This feature of Irgarol 1051s use, and its relative environmental persistence compared to other contemporary AFP booster biocides, makes it an ideal chemical tracer for boat-derived contamination. Diuron is also a commonly used booster biocide, which whilst having other uses in terrestrial weed control, has been detected frequently in densely boated areas (Thomas *et al.* 2001;Lambert *et al.* 2006). These organic biocides have been the most widely used in antifoulant paints in the UK (Environment Agency 1998) and have therefore been selected for further study.

As previously described, the area selected for the present study encompasses the most popular section for boat traffic along the River Bure, Norfolk. This lowland waterway provides opportunity to sample at sites with distinctive environmental characteristics, and a range of accessibilty to navigation and boatyards. The aim of this chapter is to quantify the spatial and temporal variation in the concentration of Irgarol 1051 and diuron in water and bed sediment, as quantified by the SPE-HPLC-MS^n method described in chapter 3. Also aimed is to determine whether a gradient in AFP biocide contamination exists in relation to the degree of exposure to boating activity. Factors such as the source, fate and transport of the antifoul biocides are discussed to assist interpretation of the spatial distribution and partitioning between aqueous and solid phases of such organic biocides. Data for the structurally similar agriculturally derived *s*-triazine atrazine and the phenylurea herbicide isoproturon are also included.

As transportation of contaminants within the environment can lead to pollution effects occurring away from the source, the spatial distribution of contaminants needs to be known for effective risk assessment and management. Quantification of organic antifoul biocide concentrations along the River Bure waterway therefore aims to determine relative exposure levels from boating activity at sites that may be considered unconnected to and uninfluenced by such activities. This study of Irgarol and diuron contamination in sediment and water within a freshwater system builds upon previous AFP biocide research. Sampling at the catchment scale, within shallow lake basins and in areas remote from navigation represents a valuable source of data for AFP biocides in previously understudied aquatic environments.

Inputs of the antifoul biocide TBT no longer occur from painted boat hulls in the Broads following the 1987 ban on its usage on boats <25 m in length. However during its period of peak usage, it was shown to be widely distributed in the dissolved phase along the River Bure (Waite *et al.* 1989). The quantification of contemporary organic antifoul biocides provides an opportunity to study contaminants that have the same source to the environment as TBT inputs had. This approach allows study of the physical processes that influence antifoul biocide spatial distribution within this area. Sharing the same input source allows some analogies to be drawn between the spatial and temporal variation of the tributyltin and organic booster biocides. Similarities in the seasonal concentration trends of organic antifoul biocides and TBT have been observed by other workers (Albanis *et al.* 2002), but there are of course some differences in their physico-chemical properties and environmental fate. These factors are discussed fully in relation to TBT distribution in Chapter 5.

Data presented in this chapter includes results of the online SPE-LC-MS^n analysis of organic biocides performed on water and sediment samples from the sites detailed in Table 2.1. This chapter focuses on investigating the influence of boating activity and hydrology on the concentration of contemporary biocides in the River Bure waterway. Results of the quarterly water-sampling programme are reported, with analysis of seasonal changes in biocide concentration. Surface sediment characterisation and biocide concentration results from the wider spatial study are statistically analysed to determine influences upon the water-solid partitioning process. The extent of partitioning between the water and bed sediments is explored using this spatial dataset and is compared to results from other contaminated sites. A detailed study of the distribution of organic biocides within a

single site, Ranworth Broad, directly connected to the navigable waterways, is also presented. This site was chosen to investigate the spatial variation of antifoul biocide concentrations at a smaller, within-lake scale.

4.2 Results and discussion

4.2.1 Spatial distribution in the dissolved aqueous phase

Water concentrations of samples analysed for antifoul booster biocides from the River Bure and associated broads ranged from <2 - 258 ng l^{-1} for Irgarol (n = 107) and <20 - 550 ng l^{-1} for diuron (n = 92) during the study period. See Appendix 9.2 for all individual water sample organic biocide concentrations. Irgarol concentrations were within the range measured by Lambert *et al.* (2006) in 2001 along the River Bure (<1 - 1332 ng l^{-1}, n = 22), but the maximum diuron concentration found in the present study (223 ng l^{-1}) is nearly double that previously determined by these workers (<1 - 139 ng l^{-1}, n = 22).

66% of all water samples analysed (total = 107) contained detectable amounts of Irgarol, whereas only 20% contained detectable diuron. The sensitivity of the analytical method for diuron was lower than for Irgarol (Irgarol LOQ = 2 ng l^{-1}), as diuron initially had an LOQ of 50 ng l^{-1}, which was improved to 20 ng l^{-1} for the latter three sampling occasions. This difference in sensitivity between the analysed biocides has truncated the diuron data to a greater extent than for the other biocides. However, all biocide LOQs are below the 100 ng l^{-1} EU drinking water guidelines. Nevertheless, maximum diuron concentrations were of the same order of magnitude as for Irgarol.

The August 2004 water sample results (Figure 4.1) show the variation in organic antifoul biocide concentrations between the sample sites and different site types (boatyards, navigable, connected and isolated). None of the sites downstream of wastewater treatment works discharges, those isolated from the river system, or located on the river upstream of the navigational limit (u/s Horstead Mill), had detectable water concentrations of Irgarol or diuron. Absence of detectable Irgarol and diuron concentrations at these sites, especially from points downstream of WWTW discharges, provides conclusive evidence that the biocide values determined from areas exposed to boating were due to contamination from antifoul

paints. The navigable and connected broads all had quantifiable Irgarol concentrations within a similar range, 3 - 10 ng l^{-1}. The greatest variation in Irgarol concentration within a site group was within the boatyards. Of these, Barnes and Loynes (Figure 2.4) had the lowest concentrations (3 & 5 ng l^{-1} respectively), with similar values to the navigable and connected broads. The other three boatyards had higher Irgarol concentrations, 20 - 195 ng l^{-1}. Similarly, all three sites with detectable diuron concentrations during August 2004 were from sites with high levels of boating activity. They were from two boatyards, Connoisseur in Wroxham (<20 ng l^{-1}), Horning Ferry Marina in Horning (223 ng l^{-1}) and the navigable Malthouse Broad (36 ng l^{-1}), a popular boating location with numerous permanent and short-term moorings.

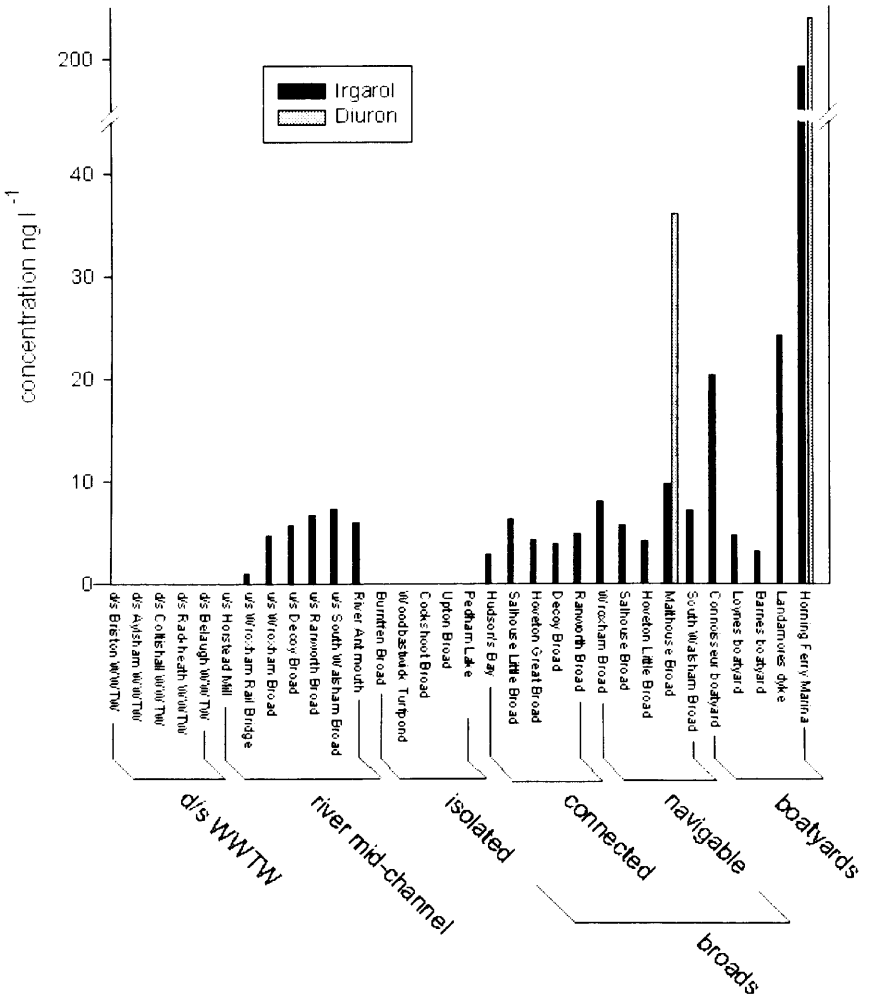

Figure 4.1 Dissolved Irgarol and diuron concentrations in the River Bure waterway, August 2004 (within each group, sites are ordered left to right by increasing distance downstream).

Overall, the highest mean concentrations of both antifoul biocides measured were within the boatyards, with decreasing amounts in the navigable and connected sites, and none detectable in the isolated sites. By far the most contaminated site for both antifoul biocides was Horning Ferry Marina. This boatyard was no larger in size than the other boatyards sampled, but did contain larger, private boats. Discussion with several boatyard owners revealed that for privately owned craft it is usual to coat the whole of the submerged hull with antifoul paint, whereas many of the hire fleet had only a thin band of antifoul paint applied at the water line.

Some of the larger boats moored at Horning Ferry Marina were sea worthy, which may have meant that they were coated with AFPs containing a higher concentration of biocides, as is common in formulations designed for marine usage. Bowman *et al* (2003) also observed higher Irgarol concentration in areas where larger vessels were moored.

4.2.2 Seasonality in dissolved organic biocide concentration

The results of the quarterly sampling are given in Figure 4.2. It is clear that Horning Ferry Marina had the highest Irgarol and diuron concentrations throughout the study period, with Landamores dyke being the second most contaminated site. No Irgarol or diuron was detected in the isolated Cockshoot Broad, or the site upstream of the navigational limit, u/s Horstead Mill. This spatial pattern of contamination provides further evidence that antifoulant paint represents the source of these compounds to the river system. Over the whole study period nearly all instances of positive diuron identification in water samples were from boatyard sites. Only the navigable sites of Wroxham Broad, Salhouse Great Broad and u/s Ranworth Broad had detectable values (>LOD) outside of boatyards in August 2003.

The seasonality of both dissolved biocides can only be seen clearly in the most contaminated boatyard sites. For Irgarol this was sites 11, 14 and 15, Connoisseur, Landamores and Horning Ferry Marina respectively. All sites had lower dissolved Irgarol concentrations in the winter months (Nov - Feb), with peaks in the summer. For diuron, Horning Ferry Marina (site 15), was the only location that had quantifiable concentrations, which displayed any distinct seasonal variation. At this site and Landamores (site 14) diuron was detected in the winter months, but at levels below the LOQ. A similar seasonal pattern of Irgarol and diuron concentrations in this most

contaminated boatyard is supportive of them having the same source to the environment. No correlation was found between dissolved biocide concentrations and the measured water quality parameters pH, temperature, electrical conductivity, total suspended solids or chlorophyll *a*.

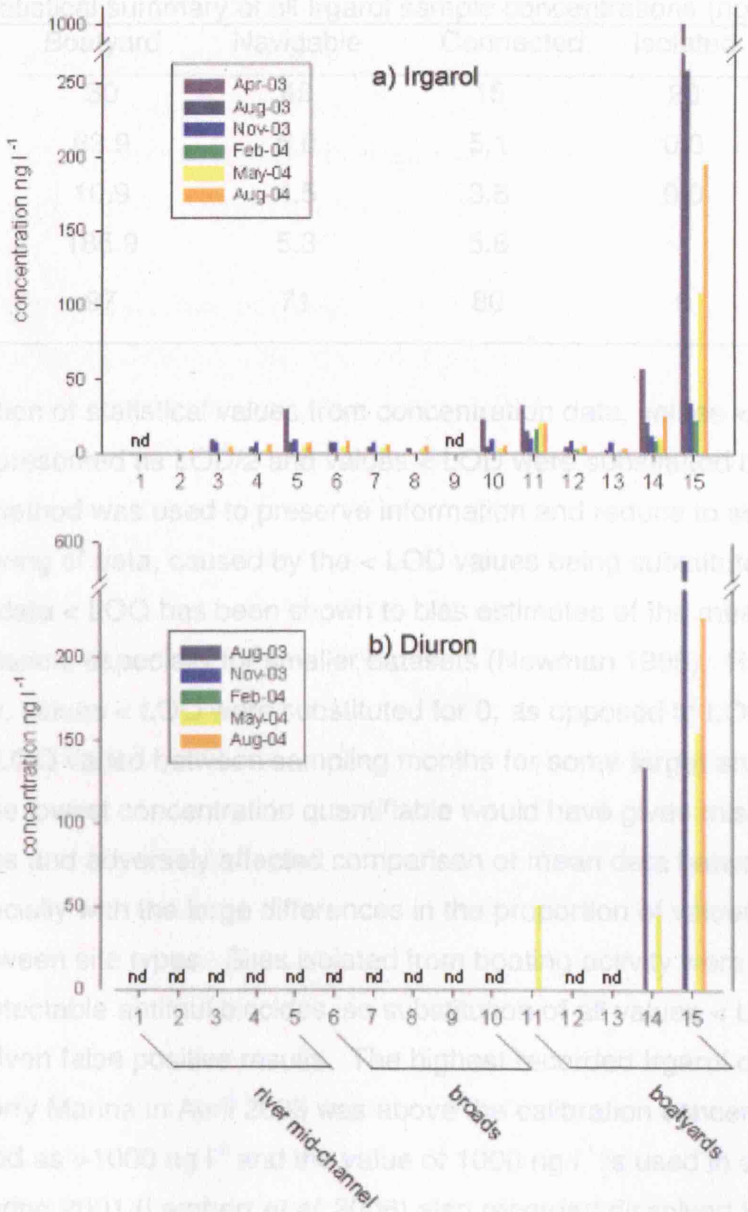

Figure 4.2 Seasonality in aqueous a) Irgarol and b) diuron concentrations (nd – not detected)

Site key: 1 – u/s Horstead Mill; 2 – u/s Wroxham Rail Bridge; 3 – u/s Wroxham Broad; 4 – u/s Decoy Broad; 5 - u/s Ranworth Broad; 6 – Wroxham Broad; 7 – Salhouse Great Broad; 8 – Hoveton Great Broad; 9 – Cockshoot Broad; 10 – Ranworth Broad; 11 – Connoisseur boatyard; 12 – Loynes boatyard; 13 – Barnes boatyard; 14 – Landamores dyke; 15 – Horning Ferry Marina. (for site locations see Figure 2.4)

As detection of Irgarol was more widespread than diuron during this study, the Irgarol data were used to determine the relative exposure levels between different

site types. In addition, Irgarol concentrations were most suitable to test whether the level and type of boating activity at each site influenced this contamination. Mean Irgarol concentrations for each boatyard, navigable, connected and isolated site type are given in Table 4.1.

Table 4.1 Statistical summary of all Irgarol sample concentrations (ng l^{-1})

	Boatyard	Navigable	Connected	Isolated
n	30	42	15	20
Mean	63.9	5.0	5.1	0.0
Median	10.9	4.5	3.8	0.0
1 S.D.	185.9	5.3	5.6	-
% positive detections	97	71	80	0

In the estimation of statistical values from concentration data, values < LOQ, but > LOD were represented as LOD/2 and values < LOD were substituted by 0. This substitution method was used to preserve information and reduce to some degree the left censoring of data, caused by the < LOD values being substituted by 0. Total censoring of data < LOQ has been shown to bias estimates of the mean and standard deviation, especially for smaller datasets (Newman 1995). However in the present study, values < LOQ were substituted for 0, as opposed to LOQ/2 for example, as LOQ varied between sampling months for some target analytes. This variation in the lowest concentration quantifiable would have given mis-leading positive values and adversely affected comparison of mean data between sampling periods, especially with the large differences in the proportion of values < LOQ observed between site types. Sites isolated from boating activity were also predicted to have no detectable antifoul biocides, so substitution of all values < LOQ for LOQ/2 would have given false positive results. The highest recorded Irgarol concentration at Horning Ferry Marina in April 2003 was above the calibration concentration range, so is presented as >1000 ng l^{-1} and the value of 1000 ng l^{-1} is used in all statistical analyses. During 2001 (Lambert *et al.* 2006) also recorded dissolved Irgarol at levels >1000 ng l^{-1} in two boatyards on the River Bure, supporting the occurrence of the similarly high concentration determined in the present study. Continued input from boat hulls coated with AFPs containing Irgarol, and possibly some desorption from that bound to bed sediment, would account for such an observation over a year after the withdrawal of Irgarol as an active AFP biocide. During the following spring

and summer Irgarol concentrations at this site were not as high, possibly reflecting successful regulatory control.

Mann-Whitney tests were used to compare Irgarol concentration means between site groups. Data were non-normally distributed and had uneven variances. Results showed that the boatyard samples had significantly higher water concentrations than both the navigable and connected sites ($p = < 0.001$ and 0.003 respectively). However there was no significant difference between the navigable and connected sites ($p = > 0.05$). (Gardinali, Plasencia, and Maxey 2004) also found a similar decrease in Irgarol concentrations outside of marinas in Biscayne Bay, Florida.

Distinct temporal variation was observed in the mean Irgarol and diuron concentration data for different sampling months (Figure 4.3 a - b). The highest mean Irgarol concentration was recorded in April 2003 in both boatyard and non-boatyard sites. By August of that year concentrations had decreased, especially in the boatyards. Diuron concentrations above the method LOD were restricted to boatyard and popular boating sites, suggesting antifoul paint as the primary source of diuron within the study area. Boatyard samples collected in the winter months (Nov and Feb) had relatively lower concentrations for both Irgarol and diuron. No quantifiable diuron was measured at all in the winter months, although several sites did have detectable chromatogram peaks for diuron, but with poor S/N ratios.

This seasonal concentration pattern of antifoul biocides in River Bure boatyards is very similar to that observed in coastal marinas in the UK open to tidal action i.e. a biocide peak early in the boating season, followed by continuous detection through the summer and much reduced concentrations over the winter. For example Thomas *et al* (2001), found that the increase in biocide concentrations followed the start of the boating season in May, peaking in June and July. Irgarol concentrations were also seen to increase in the period April to July when freshly painted craft were returned to Brighton marina (Bowman *et al.* 2003), as highest leaching rates have been shown to occur just after application (Hall *et al.* 1999). The peak in late spring and summer of the antifoul biocides is also coincident with the peak in boating activity in the Broads as a whole (Hilton and Phillips 1982;George 1992). Boats coated with antifoul paint that are returned to the water in the spring are the most likely cause of the early season peaks. Bowman et al (2003) observed elevated water concentrations of Irgarol after disturbance by a sediment dredging operation,

which was hypothesised to cause re-mobilization to the water column. However, no such activities were observed on the sampling trips during the present study.

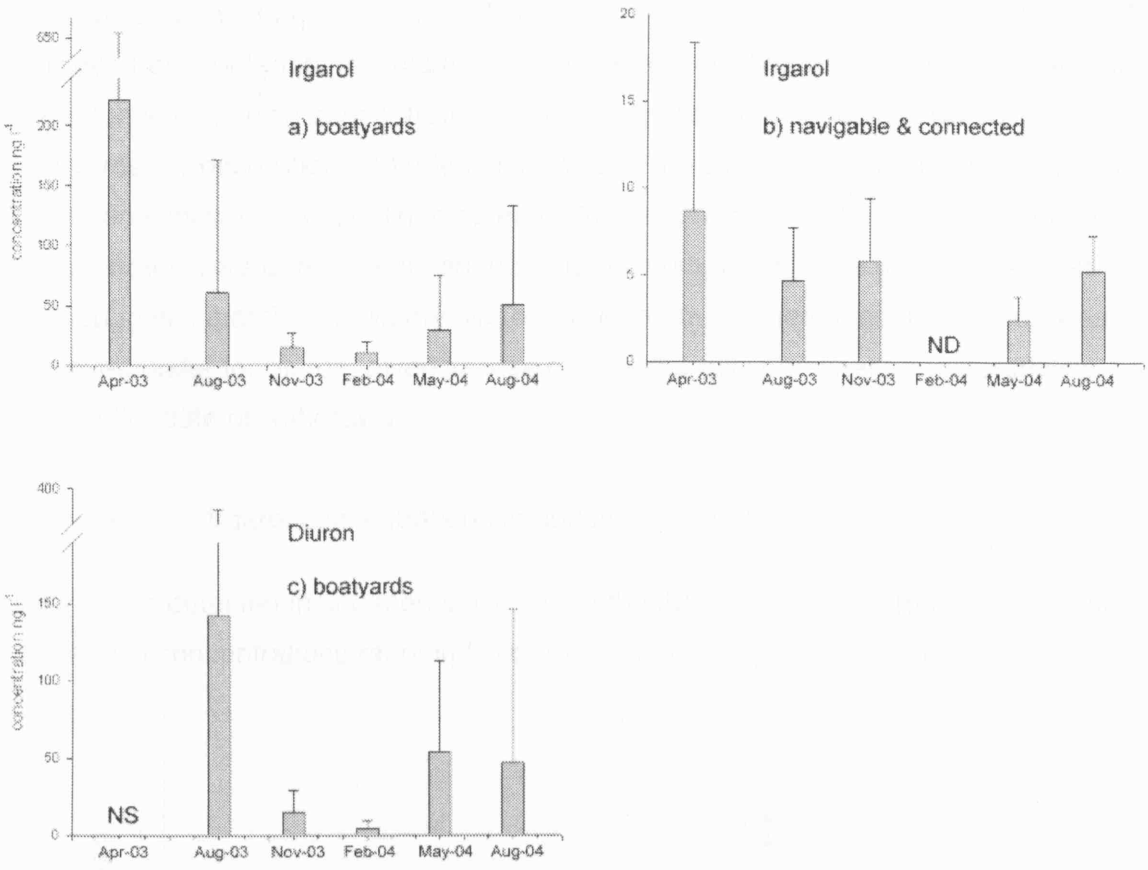

Figure 4.3 Seasonality of Irgarol (a, b) and diuron (c) water concentrations (ng l^{-1}) from different site types along the River Bure waterway (± 1 S.D.)
ND = not detected; NS = not sampled

The navigable and connected sites showed less distinct seasonal variation relative to the boatyard sites, but no Irgarol was detected from these sites in February 2004, the same time that lowest values were measured from the boatyards. Higher organic antifoul biocide concentrations were also found during and after the boating season in Greek marinas, compared to those detected before it (Albanis *et al.* 2002). In addition to lower boating activity in the winter months giving lower biocide inputs to the system, some dilution effect on concentrations during this time can also be expected from higher water levels and increased river discharge. Degradation and partitioning to the sediments will also account for some loss from the water column.

A comparison of mean August 2003 and August 2004 Irgarol values (Figure 4.3 a, b) shows only a slight concentration reduction during the summer of 2004, suggesting continued inputs from boats coated with antifoul paints containing Irgarol, despite its removal as an approved AFP biocide in November 2002. In addition, release of AFP biocides from contaminated sediments may be responsible for some of the observed persistence of biocide concentrations in the water column, especially within boatyards, as desorption and release from paint flakes incorporated in the sediment have been shown to occur (Thomas *et al.* 2003). On average August 2004 diuron concentrations were more reduced than Irgarol, compared to the previous August. This suggests that the regulatory aim of reduced environmental contamination was achieved faster for diuron, given its less environmentally persistent characteristics and earlier date of withdrawal.

4.2.3 Irgarol concentrations in bottom sediments

Irgarol was detected in sediments from 22 of the 32 sites sampled (69%) in August 2004, with concentrations ranging from 0.1 - 11.9 ng g^{-1} (LOQ = 0.1 ng g^{-1}).

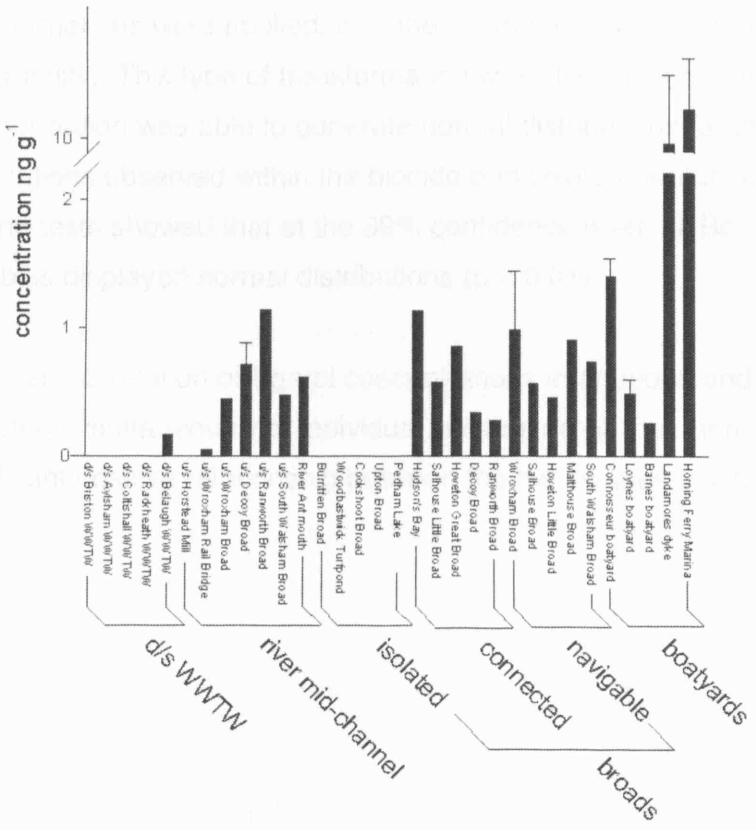

Figure 4.4 Surface sediment Irgarol concentrations along the River Bure waterway, August 2004. (± 1 S.D. for triplicate samples).

Extraction of sediment from several sites was replicated with mean values reported (see sites with error bars in Fig 4.4). In a similar pattern to the water concentrations, the spatial distribution of Irgarol in sediments reflected the level and type of boating activity at each site, hence the boatyard sites that had the highest water concentrations also had the highest sediment concentrations. Loynes and Barnes boatyards had the lowest Irgarol concentrations in both sediment and water samples. Concentrations at these two boatyards sites were more similar to those detected in the connected and navigable broads. This pattern suggests that in these two boatyards, the boats moored at the time of sampling were possibly not coated with antifoul paints containing Irgarol or diuron; a greater amount of flushing by uncontaminated river water effectively removed Irgarol from these sites; or that repainting and scrub down activities were not conducted in-situ and hence reduced the occurrence of Irgarol containing paint flakes.

Statistical analysis of the August 2004 results showed that there were no significant relationships between AFP biocide sediment concentrations and any of the measured environmental variables. Prior to correlation analysis, Box-Cox transformations were applied, with the optimal λ used to achieve a distribution closer to normality. This type of transformation was utilised as no other single transformation was able to generate normal distributions for the variety of distributions observed within the biocide and environmental variables. Anderson-Darling tests showed that at the 99% confidence level, all Box-Cox transformed variables displayed normal distributions ($p < 0.01$).

However, correlation of Irgarol concentrations in aqueous and sediment phases collected simultaneously at individual sites revealed that there was a strong and significant positive relationship between the two phases ($r = 0.891$, $p = < 0.001$).

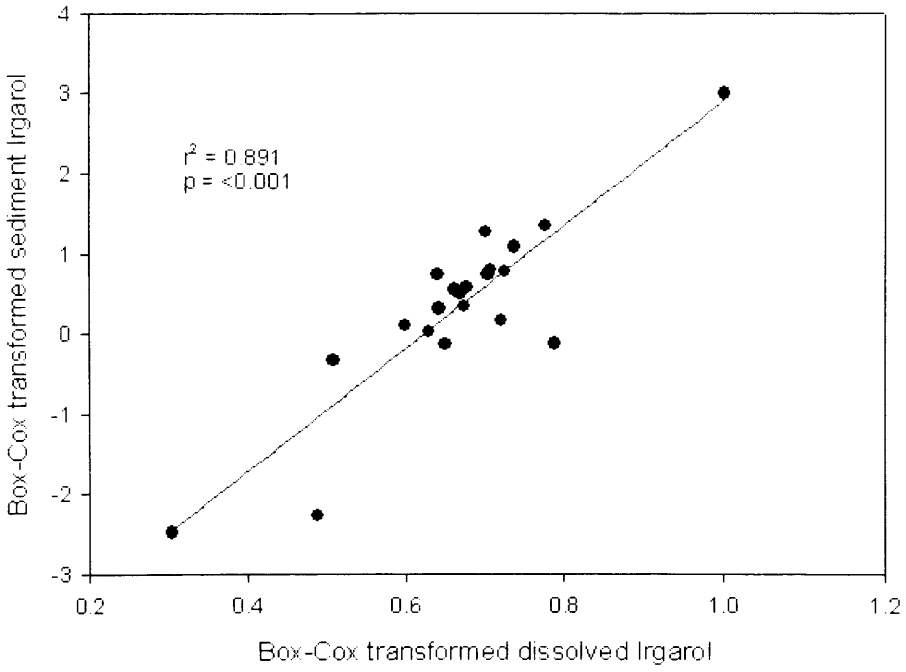

Figure 4.5 Regression showing the relationship between dissolved and sediment-bound Irgarol concentrations after Box-Cox transformation.

No Irgarol was detected in sediments from any of the isolated broads, or the river and WWTW sites upstream of the navigational limit. A small amount of Irgarol (0.2 ng g^{-1}) was quantifiable in sediment at the d/s Belaugh WWTW site, which was also the highest upstream navigable point sampled (Figure 2.6). The sample was collected by boat from sediment just downstream of where the WWTW discharge enters the main navigable river channel. The water sample collected simultaneously from this site however did not have any detectable Irgarol. Figure 2.2 shows there were relatively few boats registered upstream of Wroxham, which helps explain the Irgarol water concentration being < LOD. Inputs from the WWTW discharge itself were predicted to be zero, as no Irgarol was detected downstream of any of the other WWTW discharges sampled in the headwaters of the catchment. Boats do travel upstream of the Belaugh WWTW sample site, to moorings in the village of Coltishall. However, given the relatively low boat traffic and small number of moorings in this upstream section of the river, the input of antifoul biocides is predicted to be low. As Irgarol was detected within the sediment, but not in the water collected at the same time, this may reflect temporal variability in water concentrations, or very low contamination levels below the LOD. The biocide had clearly integrated into the sediments, via partitioning from that dissolved in the water.

This indicates that either sorption to sediment had occurred in-situ, or biocide already bound to sediment had been transported from a contamination source further upstream. The next site downstream, u/s Wroxham Rail Bridge, positioned just upstream of the Wroxham boatyard complex, also had a relatively low sediment Irgarol concentration. The amount of Irgarol in the sediments downstream of Wroxham village progressively increased, peaking at the site u/s Ranworth Broad. All the navigable and connected broads sampled (all downstream of Wroxham) had quantifiable Irgarol sediment concentrations.

Mean Irgarol sediment concentrations, from the different site types, displayed a gradient in contamination related to relative exposure to boating (Figure 4.6). It is clear the boatyards have received a large input of Irgarol relative to the other site types, whereas variance in atrazine distribution between different site types was minimal. Significantly, no Irgarol was detected in sites isolated from the navigable river but atrazine was. This suggests that sediment character was not responsible for variance in Irgarol concentration at the catchment scale, but intensity of boating activity was.

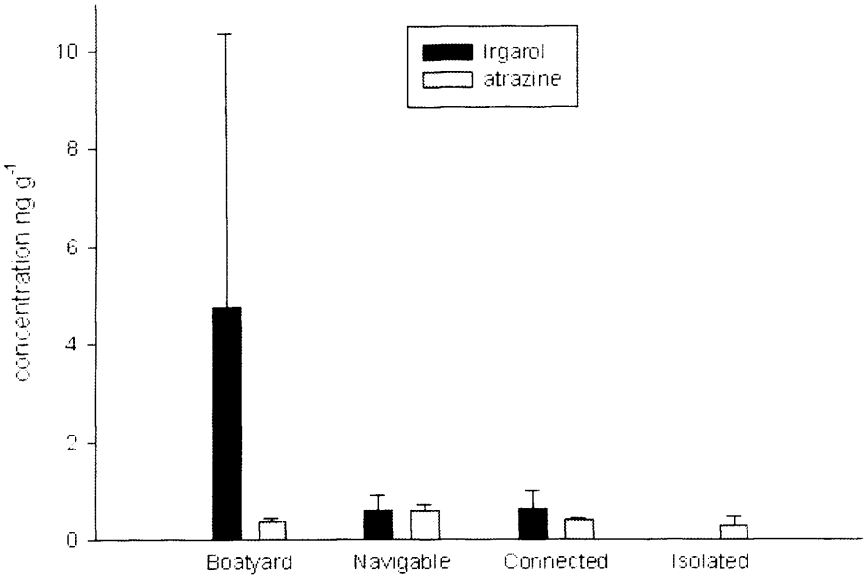

Figure 4.6 Mean Irgarol and atrazine sediment concentrations from different site types (± 1 S.D.)

Maximum concentrations of Irgarol in the sediments of the River Bure are shown to be an order of magnitude greater than the maximum dissolved concentrations (grey bars in Figure 4.6). In terms of the Irgarol concentrations reported from around the

world, those determined in the marine environment far exceed those in freshwaters. Thus to simplify the comparison with marine studies, only Irgarol concentrations reported from within the UK marine environment are presented in Figure 4.7. The one comparable freshwater study with simultaneous Irgarol concentrations determined from water and sediments was in Lake Geneva , which also showed similar concentration ranges in both compartments (Tóth *et al.* 1996). Dissolved Irgarol concentrations in the present study are greater than any of the other freshwater values reported from around the world, with the exception of the baseline data for the Broads and selected East Anglian rivers given in Lambert et al (2005).

Results from the Broads suggest that this area has a relatively high level of antifoul biocide contamination compared to other popular boating locations around the world.

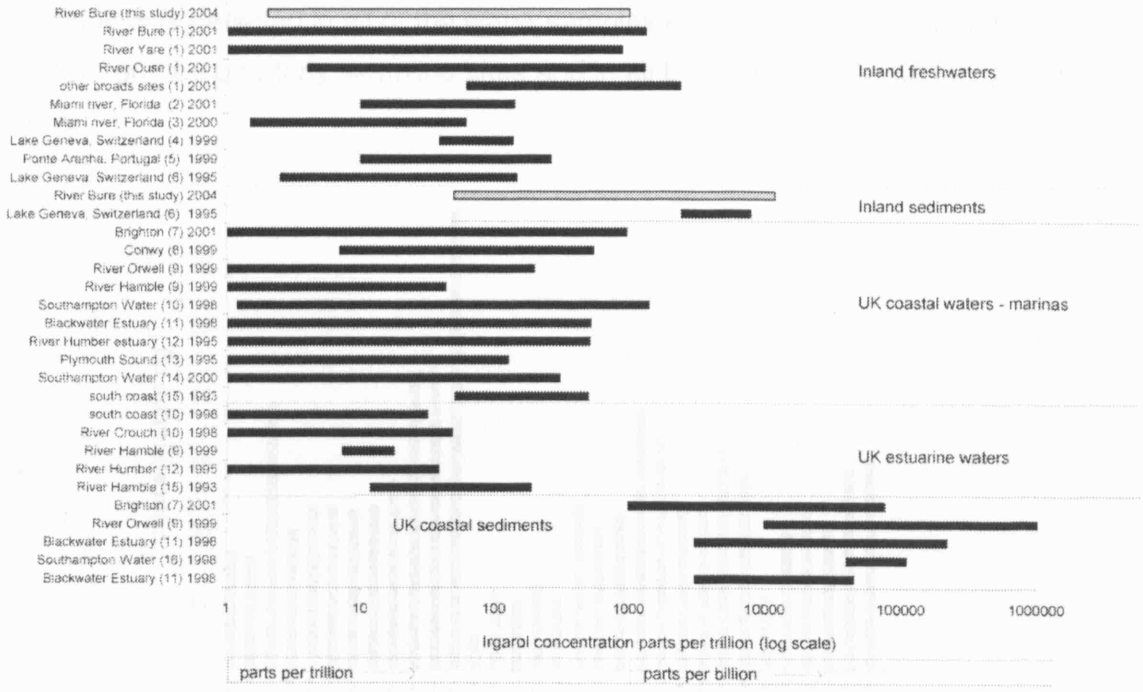

Figure 4.7 Reported Irgarol concentration ranges (scale in parts per trillion, grey bars = results from the present study).

References: 1 -(Lambert *et al.* 2006); 2 - (Gardinali *et al.* 2004); 3 - (Gardinali *et al.* 2002); 4 - (Nyström *et al.* 2002b); 5 - (Almeida Azevedo *et al.* 2000b); 6 - (Tóth *et al.* 1996); 7 - (Bowman *et al.* 2003); 8 - (Sargent *et al.* 2000); 9 - (Boxall *et al.* 2000); 10 - (Thomas *et al.* 2001); 11 - (Voulvoulis *et al.* 2000); 12 - (Zhou *et al.* 1996); 13 - (Scarlett *et al.* 1997); 14 - (Thomas *et al.* 2002); 15 - (Gough *et al.* 1994); 16 - (Thomas *et al.* 2000).

Maximum water concentrations in the Broads are within the same order of magnitude, and slightly higher, than those reported from UK coastal marinas. Maximum coastal marina dissolved concentrations are roughly an order of magnitude higher than samples collected from boated estuarine sites away from permanent moorings. This magnitude of difference is similar to that observed between the concentrations measured in the most contaminated River Bure boatyards and the levels in navigable and connected sites. The reasons for differences in AFP contamination in marine and freshwater environments will be discussed in section 4.2.5.

4.2.4 Concentrations of agriculturally derived herbicides in water and sediments

Atrazine was detected in all of the water samples except one (Cockshoot, February 2004) from the quarterly sampling sites and in all the extended August 2004 survey sites.

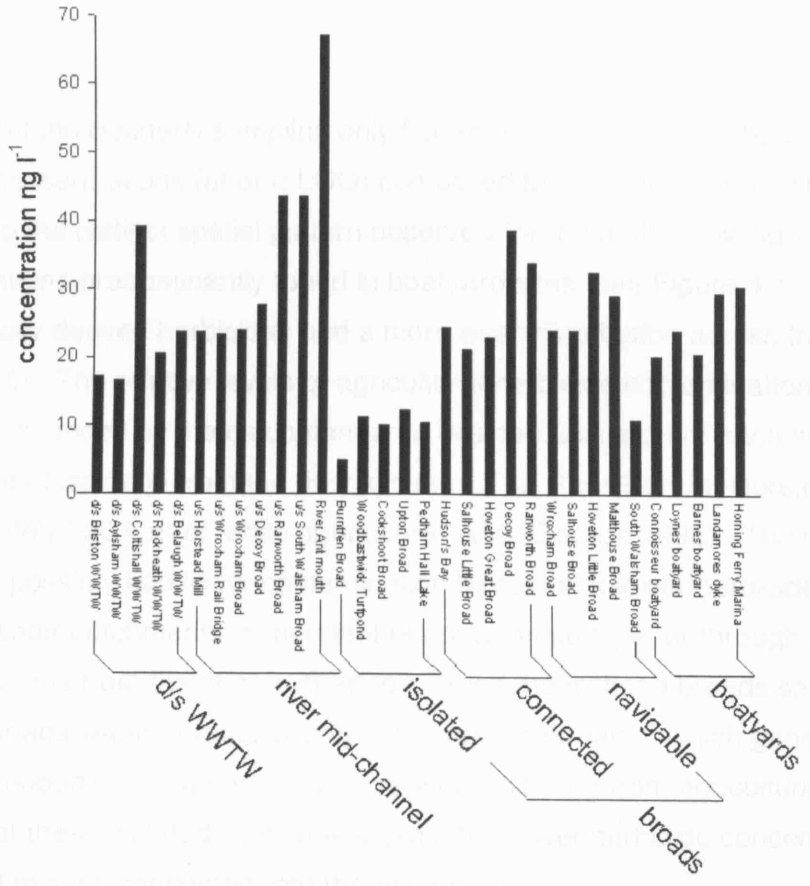

Figure 4.8 Dissolved atrazine concentrations along the River Bure waterway during August 2004

During August 2004 quantifiable atrazine concentrations from the 32 sites sampled were in the range 10 - 67 ng l^{-1}, with a mean of 25 ng l^{-1} (S.D. ± 12). Isoproturon was not found at quantifiable concentrations during August 2004, but was frequently detected during February and May 2004, with a quantifiable concentration range of 14 - 92 ng l^{-1} (see Appendix 9.2).

During the August 2004 survey, the lowest atrazine concentrations were observed in the isolated broad sites and in South Walsham Broad. Concentrations measured along the river channel (sites d/s Briston WWTW to u/s South Walsham Broad) tended to increase with distance downstream, with only the site d/s Coltishall WWTW being out of step with this pattern (Figure 4.8). The sample taken from the River Ant mouth, upstream of where it entered the River Bure, gave the highest atrazine concentration during this month. The River Ant drains a predominantly arable agricultural area, similar to the River Bure catchment (George 1992), but this data suggests that a slightly larger amount of atrazine may be leached from the Ant catchment, possibly due to greater inputs per area drained and/or more permeable soils.

Throughout the quarterly sampling only Cockshoot Broad (site 9) had markedly lower atrazine concentrations (at or < LOQ) compared to the other sample sites. In contrast to the distinct spatial pattern observed for the antifoul biocides, with highest concentrations predominantly found in boatyard sites (see Figure 4.1), the agriculturally derived herbicides had a more even distribution across the study area (Figure 4.8). The relative levels of agricultural herbicide contamination appeared to be more influenced by the catchment area drained upstream of each sample site and the degree of isolation from the river system. The River Bure at Horstead Mill drains approximately 330 km^2 of fertile agricultural land (George 1992). The most upstream river sites possibly had lower contamination because of lower herbicide application in the headwater catchments, dependant upon land use type, or through the distance of arable areas from the stream channels. Also the isolated broads sampled, either had no surface water inflows, or only small feeder streams, which generally passed through wooded or fen areas. The increased buffering from agricultural herbicide residues at these isolated sites may explain the lower herbicide concentrations compared to sites connected with the main river.

In contrast to the antifoul biocides, boatyard atrazine concentrations were not significantly greater than the other sites. No commonality in source could therefore be ascribed between the agricultural herbicides and the antifoul biocides. Within the quarterly sampled mid river channel and boatyard sites, Figure 4.9 shows that atrazine concentrations were recorded on each sampling occasion. In turn this suggests that minimal local atrazine inputs to this section of river. Such lack of gross variation indicates that the bulk of the atrazine load was probably delivered in solution from further upstream in the catchment area, with individual sites having similar loads.

Transport in solution is the most likely route, due to atrazine's relatively high solubility of 33 mg l^{-1}, see Table 9.3 (Appendix 9.1). The solubility of atrazine also makes it a common contaminant of groundwater (Wauchope *et al.* 1992), which may explain why quantifiable concentrations were also observed in even the most hydrologically isolated of sites (Figure 4.8). Figure 4.9b shows that relatively lower isoproturon concentrations were detected in the most upstream river mid-channel sites (1-3) when compared to the lower navigable sites. In general there was no significant difference in concentrations between the river, broad or boatyard sites. However, no isoproturon was detected in any samples from the isolated Cockshoot Broad, suggesting no, or minimal, inputs within this broad's small, mainly wooded catchment area.

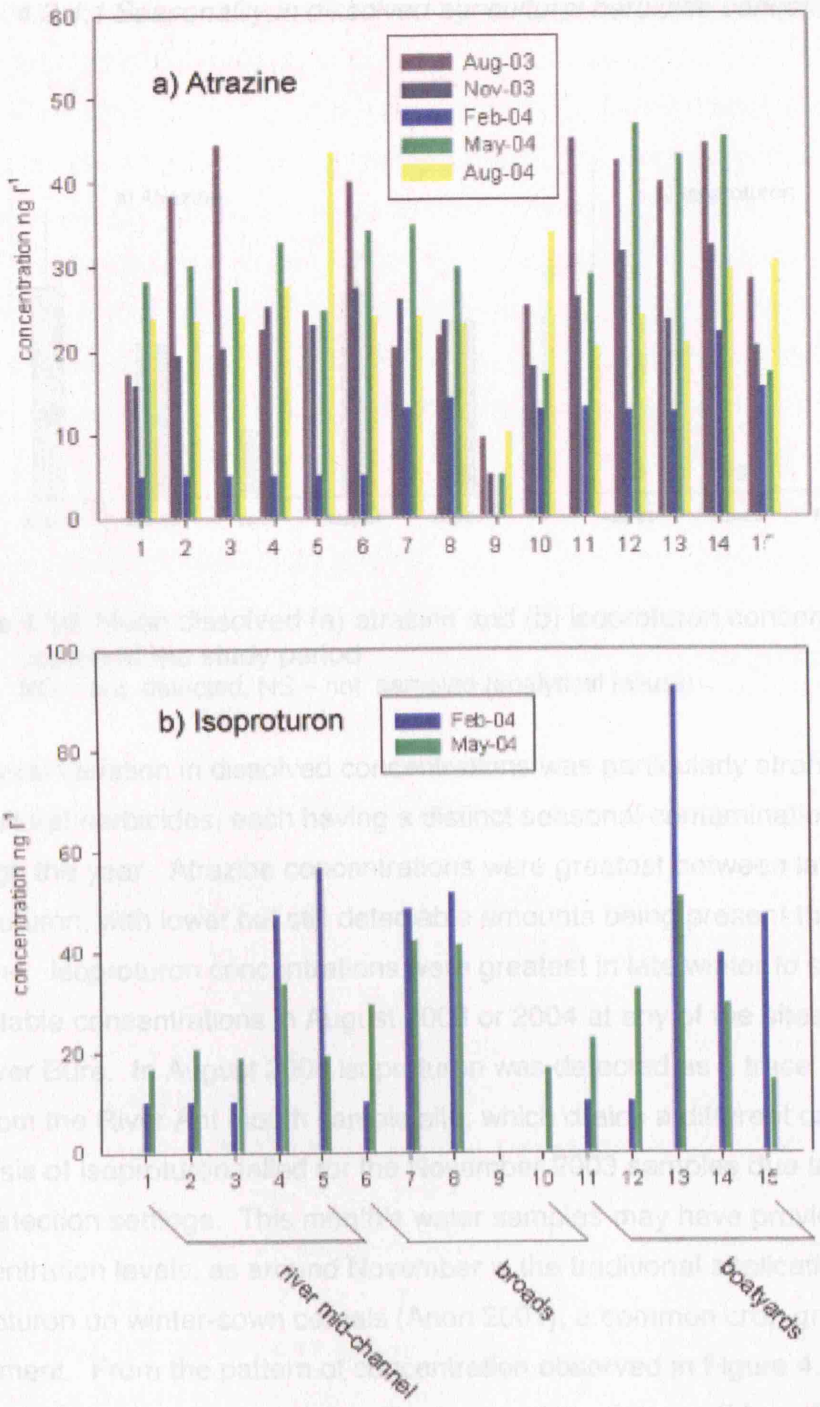

Figure 4.9 Seasonal variation in dissolved (a) atrazine and (b) isoproturon concentrations along the River Bure waterway.

Site key: 1 – u/s Horstead Mill; 2 – u/s Wroxham Rail Bridge; 3 – u/s Wroxham Broad; 4 – u/s Decoy Broad; 5 - u/s Ranworth Broad; 6 – Wroxham Broad; 7 – Salhouse Great Broad; 8 – Hoveton Great Broad; 9 – Cockshoot Broad; 10 – Ranworth Broad; 11 – Connoisseur boatyard; 12 – Loynes boatyard; 13 – Barnes boatyard; 14 – Landamores dyke; 15 – Horning Ferry Marina.

4.2.4.1 Seasonality in dissolved agricultural herbicide concentrations

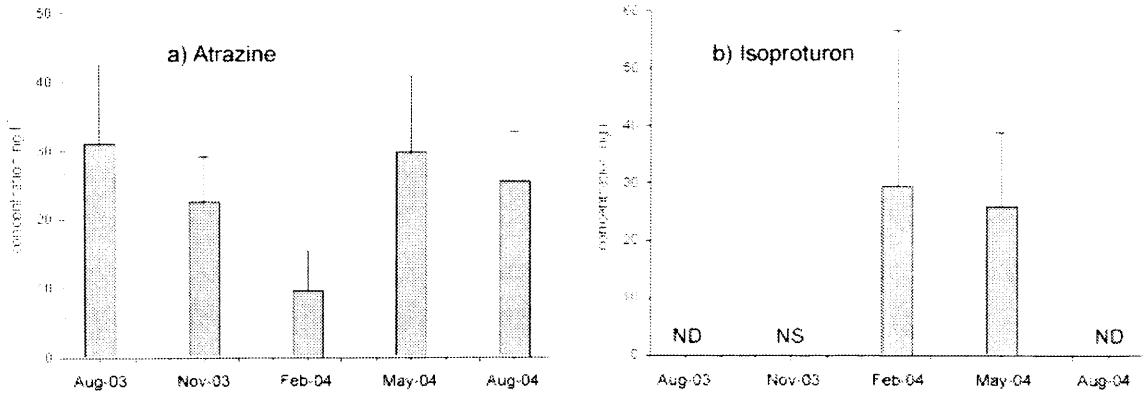

Figure 4.10 Mean dissolved (a) atrazine and (b) isoproturon concentrations over the course of the study period
ND – not detected, NS – not sampled (analytical failure)

Temporal variation in dissolved concentrations was particularly strong for both of the agricultural herbicides, each having a distinct seasonal contamination pattern through the year. Atrazine concentrations were greatest between late spring and late autumn, with lower but still detectable amounts being present through the winter months. Isoproturon concentrations were greatest in late winter to spring, with no detectable concentrations in August 2003 or 2004 at any of the sites connected to the river Bure. In August 2004 isoproturon was detected as a trace amount (< 10 ng l^{-1}), from the River Ant mouth sample site, which drains a different catchment area. Analysis of isoproturon failed for the November 2003 samples due to an error in the MS detection settings. This month's water samples may have provided quantifiable concentration levels, as around November is the traditional application time for isoproturon on winter-sown cereals (Anon 2001), a common crop grown in the catchment. From the pattern of concentration observed in Figure 4.10, it is suggested that isoproturon enters the river system via run-off from fields during the winter months with further additions to the load arising from applications made in the spring.

These observed seasonal patterns in dissolved herbicide concentrations in the River Bure waterway not only reflect the application times of herbicides to agricultural areas. Previous research has also suggested that the proximity of crop fields; hydrological connectivity to the main watercourse; the pattern and intensity of rainfall

in the catchment; and the degradation rate of the herbicides, are important factors influencing observed patterns of concentration (House *et al.* 1997;Garmouma *et al.* 1998). The use of atrazine for the pre and early post-emergence control of annual and broad-leaved weeds and perennial grasses, especially in maize and sweetcorn (Whitehead 2001), led to an observed peak in atrazine concentrations in the River Marne, France between April and July (Garmouma *et al.* 1998). In the UK isoproturon is mainly applied around November, in-line with its application period on winter cereals (Whitehead 2001). However, Garmouma *et al* (1998) found that isoproturon concentrations in French rivers peaked between March and April, as spring application was more common in their study area. The seasonality in concentrations of atrazine and isoproturon in the River Bure appeared to follow the pattern of application to locally grown crops. The relative persistence of atrazine, being detected through the year, may be a function of its lower solubility and longer half-life compared to isoproturon (see Table 9.3), which appears to be below detection in the summer.

4.2.4.2 Sediment-bound concentrations of agriculturally derived herbicides

Sediment atrazine concentrations were quantifiable from 29 (91%) of the 32 sites sampled during the August 2004 spatial survey. Concentrations at individual sites ranged from 0.3 – 1.0 ng g^{-1}, with a mean of 0.5 ng g^{-1} (± 0.2). The three sites where sediment atrazine was < LOD were isolated sites, which also had the lowest dissolved concentrations. The sites downstream of WWTWs in the headwaters and the most upstream river channel sites also had slightly lower sediment concentrations than the other sites along the middle section of river, similar to the spatial pattern observed in dissolved concentrations. However no significant relationship was found between the concentration of dissolved atrazine and that bound to sediments. Atrazine spatial distribution in freshwater sediments has been shown to be largely determined by sediment organic carbon content (Gao *et al.* 1997), but at the spatial scale analysed in the present study, no such relationship was found.

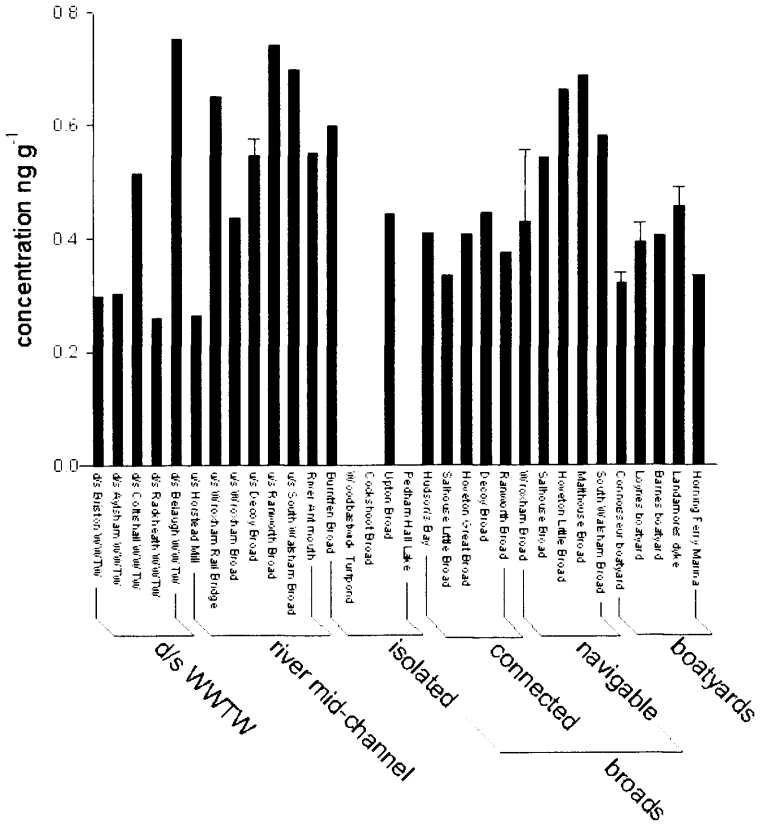

Figure 4.11 Atrazine sediment concentrations, August 2004 (± 1 S.D. for triplicate analysed samples)

4.2.5 Water - sediment biocide interactions

In the present study the triazine compounds Irgarol and atrazine were successfully quantified in the dissolved phase and from the bed sediments of the River Bure waterway. The predominant mechanism determining partitioning of such hydrophobic organic contaminants from the dissolved phase to sediment and soil has been shown to be sorption to organic matter (Gao *et al.* 1997;Voulvoulis *et al.* 2002;Park *et al.* 2003). However other environmental factors have also been shown to influence the distribution of triazine compounds between aqueous and sediment phases, for example clay minerals act as effective sorbents for atrazine (Laird *et al.* 1994). In the case of antifoulant biocides, with their often localised inputs, the specific characteristics of the sample site, for example the density of boats and the water residence time, have also had a major influence on the relative concentrations between sediments and overlying water (Biselli *et al.* 2000). Given the high amount of variablity in the sediment types and different exposure levels to triazine biocides, between the sampled sites, analysis of the spatial data to determine whether

sediment character or water quality influenced biocide partitioning to the sediment was performed. Firstly, a comparison of Irgarol and atrazine interactions with sediment and other measured environmental variables is presented, in order to inform later discussion.

Reported log K_{oc} (carbon normalised sediment-water partition coefficient) values are within a higher range for Irgarol (2.4 – 4.9) compared to atrazine(2.1 – 2.4) (see Appendix 9.1). However at the spatial scale and sediment organic levels sampled in this study, a positive relationship was not found between biocide sediment concentration and sediment organic matter for either triazine biocide. For this dataset weak but significant negative relationships existed between Irgarol sediment concentration and percentage organic matter (r^2 = 0.540, p = 0.009) and percentage clay (r^2 = -0.633, p = 0.002).

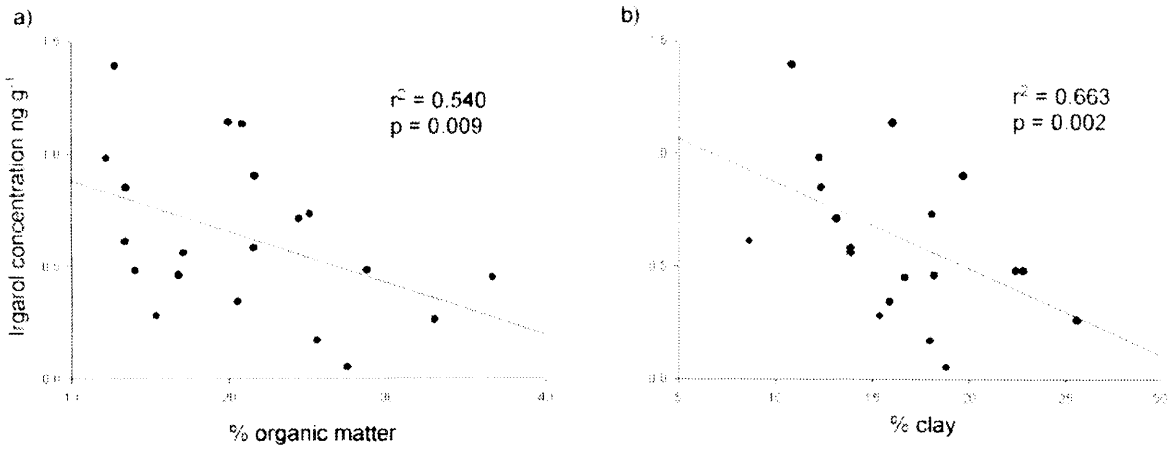

Figure 4.12 Relationship between Irgarol sediment concentration and a) organic matter and b) clay content. n = 20.

To achieve a normal distribution within the Irgarol data prior to statistical analysis, the two boatyard samples with the highest Irgarol concentrations were removed as outliers, defined here as concentrations being > 2 S.D. than the mean Irgarol concentration for all samples.

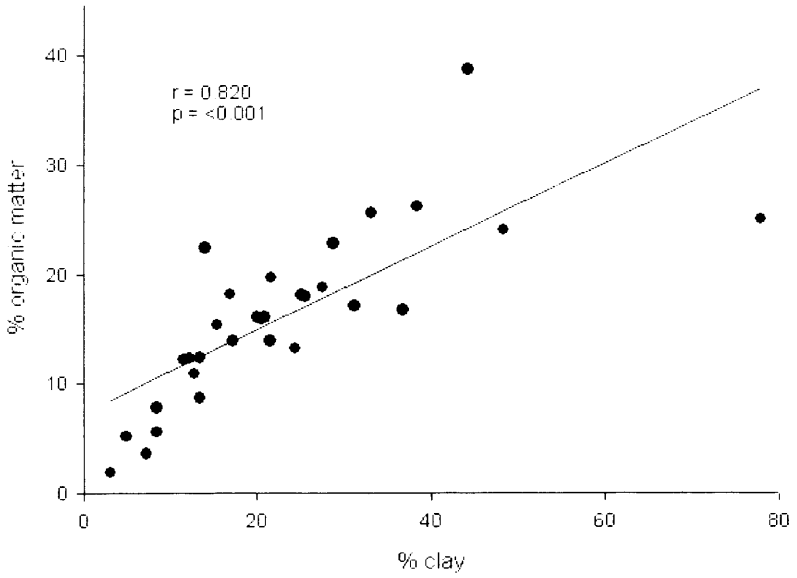

Figure 4.13 Relationship between sediment percentage organic matter and clay fraction. n = 32.

Removal of the two outlier points actually reduced the agreement with the line of best fit when compared to the full dataset. However the significant negative relationships between Irgarol sediment concentration and organic matter and clay were preserved. Organic matter and clay were found to be co-variable, with a significant positive relationship ($r = 0.820$, $p = < 0.001$)(Figure 4.13).

Given the observed pattern of negative relationships between Irgarol sedment concentrations and sediment character given in Figure 4.12, Irgarol partitioning to either organic matter or clay minerals does not appear to determine the relative sediment concentrations measured between individual sites. However, for atrazine sediment concentrations, a significant but weak positive relationship existed with the proportion of the clay fraction within the sediments sampled ($r = 0.411$, $p = 0.033$)(Figure 4.13).

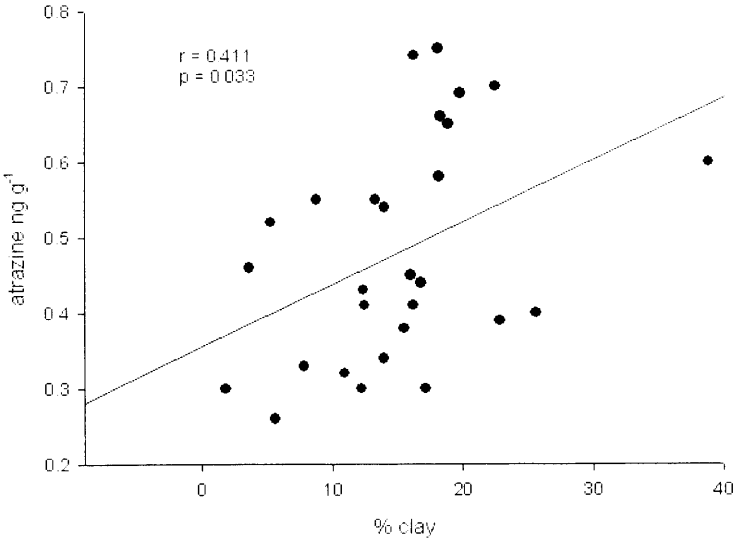

Figure 4.14 Relationship between atrazine sediment concentrations and sediment percentage clay content. n = 32.

This observation suggests that the greater surface area of clay particles, compared to other sediment size fractions, had an influence on atrazine sorption, as in general, greater atrazine sediment concentrations were found in areas of higher clay content. Laboratory studies have shown that clay minerals have a role to play in triazine sorption, but to a lesser extent than the organic matter present in natural sediments (Spark and Swift 2002). The sorption properties of solid phase organic matter toward non-polar and weakly polar compounds, such as the biocides analysed in the present study, have been found to be relatively unaffected by variation in pH and ionic strength (Schwarzenbach, Gschwend, and Imboden 2003). Accordingly, within the River Bure data no significant relationships were found between either of the triazine compounds sediment concentration and the directly measured pH or electrical conductivity. In their laboratory studies on antifoulant biocide partitioning to iron hydroxide, Voulvoulis *et al* (2002) also found that neither pH nor salinity significantly affected Irgarol sorption to this solid phase material.

The distribution of triazine biocides between water and sediment compartments measured at the river catchment scale clearly does not follow simple model predictions, such as the observed increase in sediment bound biocide with increasing sediment organic carbon content (Gao *et al.* 1997). However, atrazine sediment concentration does appear to be weakly related to the proportion of clay fraction in sediments within the study area. Other factors that influence biocide

sediment concentrations other than in-situ partitioning include, input of contaminated suspended material transported from elsewhere and biocide loss to the overlying water through processes that increase desorption rates, such as sediment resuspension (Comber *et al.* 2001). The localised nature of areas of intense antifoul biocide inputs, such as that observed within boatyards, also makes determination of the factors influencing partitioning extremely difficult, especially when sampling at the catchment scale, as these individual heavily contaminated sites significantly skew the data. For example the two highest sediment Irgarol concentrations were from sites that had the lowest percentage of organic matter. This observation suggests that, for Irgarol at least, intensity of input from boats coated with AFP is the dominant influence on the spatial distribution in sediments at the catchment scale. The similar spatial distribution pattern of dissolved Irgarol concentrations supports this theory. Overall, sample site sediment characteristics explain little of the distributional variability in the Irgarol sediment concentration dataset.

Redundancy analysis (RDA) was performed on the whole August 2004 dataset with the sediment biocide concentrations as response variables and sediment character and site type as predictor variables. The site types, isolated, connected, navigable, and boatyard, were factored into the analysis as dummy variables. This involved creation of a numeric variable for each site type. Locations included within that site type were expressed as positive, by ascription of the value of 1, with all other locations not in that site type, expressed as 0. Figure 4.15 shows the ordination triplot for Irgarol and atrazine sediment concentrations. The variation in the dataset explained by axis 1 was 43.1% and by axis 2 was 25.2%.

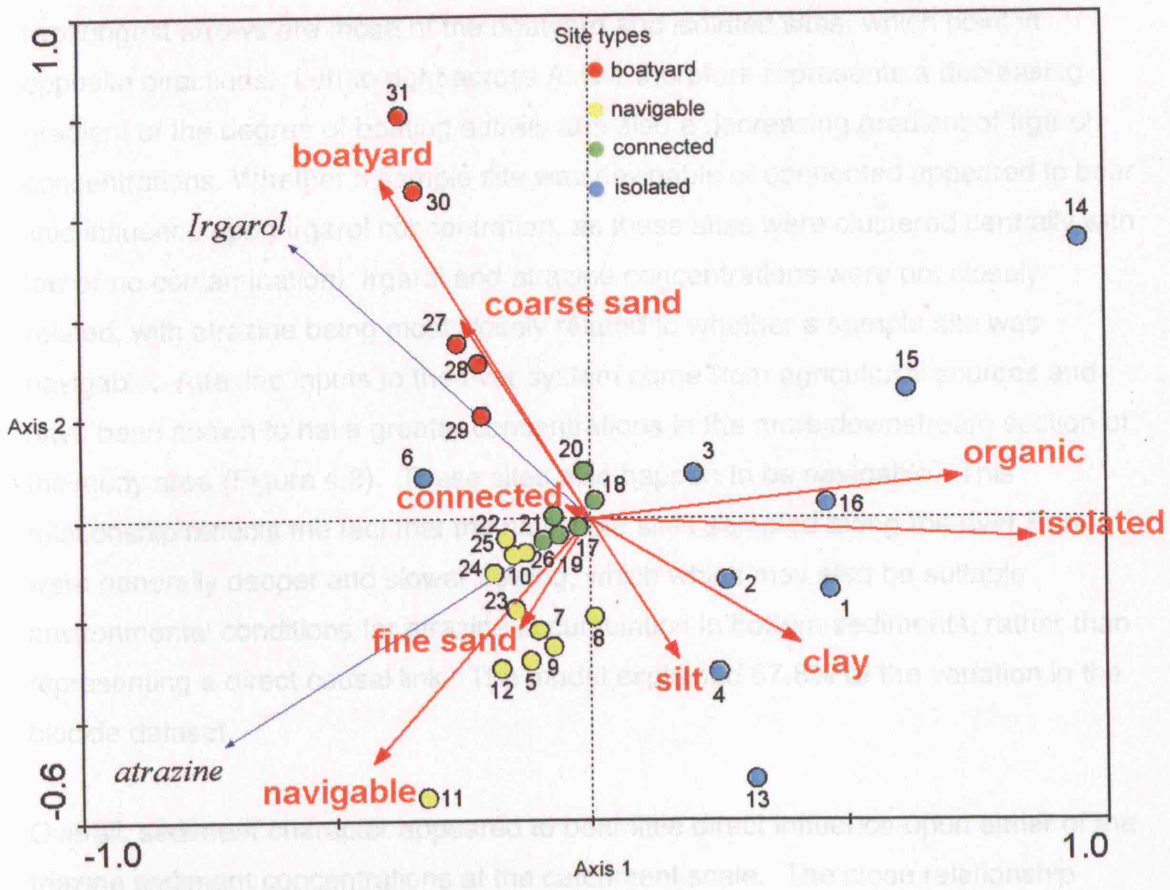

Figure 4.15 RDA triplot of triazine sediment concentrations with sediment characteristics and site type.

Site key: 1 - d/s Briston WWTW; 2 – d/s Aylsham WWTW; 3 – d/s Coltishall WWTW; 4 – d/s Rackheath WWTW; 5 – d/s Belaugh WWTW; 6 – u/s Horstead Mill; 7 – u/s Wroxham Rail Bridge; 8 – u/s Wroxham Broad; 9 – u/s Decoy Broad; 10 - u/s Ranworth Broad; 11 – u/s South Walsham Broad; 12 – River Ant mouth; 13 - Burntfen Broad; 14 – Woodbastwick Turfpond; 15 - Cockshoot Broad; 16 – Upton Broad; 17 Hudson's Bay; 18 – Salhouse Little Broad; 19 - Hoveton Great Broad; 20 – Decoy Broad; 21 - Ranworth Broad; 22 - Wroxham Broad; 23 – Salhouse Great Broad; 24 – Hoveton Little Broad; 25 – Malthouse Broad; 26 - South Walsham Broad; 27 - Connoisseur boatyard; 28 – Loynes boatyard; 29 – Barnes boatyard; 30 – Landamores dyke; 31 – Horning Ferry Marina. Analysis of Pedham Lake sample failed.

Interpretation of the RDA triplot shows that Irgarol sediment concentration was most closely correlated to whether a site was a boatyard, as shown by the similar angle of the Irgarol and boatyard arrows. The boatyards sample site positions (red circles, 27-31) in the ordination space extend along this direction towards the top left of the diagram. On the right-hand side of Axis 1 the broad and river sample sites (1-4 & 13-16) isolated from boating activity were effectively separated from the other samples in this analysis. These sites also tended to be relatively uncontaminated by both of the triazine compounds. The length of the arrows representing the environmental variables shows the relative amount of variance in the response data (sediment biocide concentration) that each environmental variable explained. The

two longest arrows are those of the boatyard and isolated sites, which point in opposite directions. Left to right across Axis 1 therefore represents a decreasing gradient of the degree of boating activity and also a decreasing gradient of Irgarol concentrations. Whether a sample site was navigable or connected appeared to bear little influence upon Irgarol concentration, as these sites were clustered centrally with low or no contamination. Irgarol and atrazine concentrations were not closely related, with atrazine being most closely related to whether a sample site was navigable. Atrazine inputs to the river system come from agricultural sources and have been shown to have greater concentrations in the more downstream section of the study area (Figure 4.8). These sites also happen to be navigable. This relationship reflects the fact that the navigable sites sampled along the river Bure were generally deeper and slower flowing, which which may also be suitable environmental conditions for atrazine accumulation in bottom sediments, rather than representing a direct causal link. The model explained 67.8% of the variation in the biocide dataset.

Overall, sediment character appeared to bear little direct influence upon either of the triazine sediment concentrations at the catchment scale. The close relationship between boatyard sites and the coarse sand fraction of sediments within these sites reflects the highly modified and managed nature of substrate in these locations. Conversely, the isolated sites (blue circles in Figure 4.15) were most closely linked to the organic matter fraction. The position of Site 6 (u/s Horsted Mill) in Figure 4.15 was skewed by the very high coarse sand fraction at this site (83%). The relatively high sediment organic levels at most of the isolated sites may be due to greater water retention times from the lack of flushing by river water. These landscape characteristics therefore lead to decomposition and accumulation of organic matter in-situ and relatively small inputs of eroded mineral sediment from the catchment. Left to right along the bottom of Figure 4.15 also appears to represent a gradient of decreasing mean particle size, ranging from sand to clay.

As the boatyard samples with relatively high Irgarol sediment concentrations (n = 5) and the headwater and isolated broad sites with no Irgarol contamination (n = 9) exerted a large amount of influence upon the sample site distribution in the ordination space, a similar analysis was performed but excluding results from these two site types (Figure 4.16).

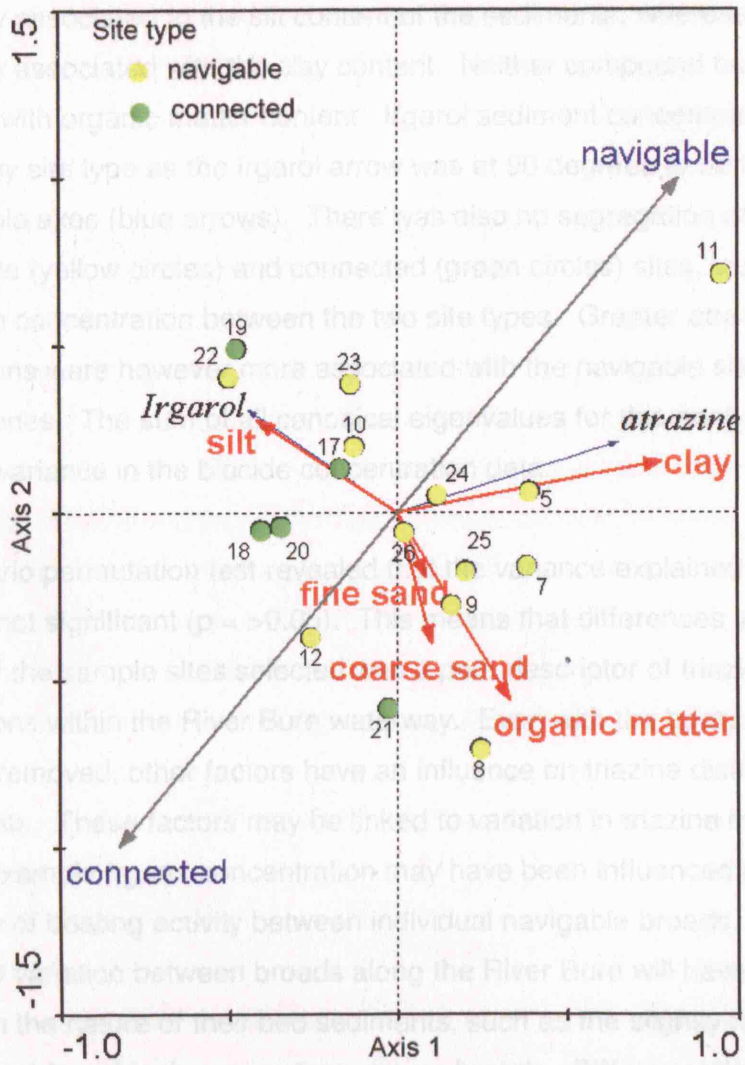

Figure 4.16 RDA triplot of triazine sediment concentration and sediment character. (same sample site numbering as Figure 4.15)

The remaining site type variables, connected and navigable, were also only plotted as supplementary variables rather than influencing the sample sites position in the ordination. This resulted in an analysis focussed on the distribution of the triazine biocides, as influenced by sediment character.

In this analysis, the association of Irgarol sediment concentration and the proportion of coarse sand is no longer evident (as in Figure 4.15), presumably as this fraction was greatest in a few of the heavily contaminated boatyard samples which have been omitted here. Of the sediment characteristics measured, the proportion of clay and organic matter explained the greatest percentage of variation in the distribution of the sample sites in the ordination (longest arrows). Irgarol concentration was

most closely associated to the silt content of the sediments, whereas atrazine was most closely associated with the clay content. Neither compound had a positive association with organic matter content. Irgarol sediment concentration was not influenced by site type as the Irgarol arrow was at 90 degrees to both the connected and navigable axes (blue arrows). There was also no segregation of the positions of the navigable (yellow circles) and connected (green circles) sites, indicating little difference in concentration between the two site types. Greater atrazine concentrations were however more associated with the navigable sites than connected ones. The sum of all canonical eigenvalues for this analysis explained 28% of the variance in the biocide concentration data.

A Monte Carlo permutation test revealed that the variance explained by Axis 1 in the model was not significant ($p = >0.05$). This means that differences in the sediment character of the sample sites selected was a poor descriptor of triazine sediment concentrations within the River Bure waterway. Even with the boatyard and isolated site results removed, other factors have an influence on triazine distribution within the sediments. These factors may be linked to variation in triazine input between sites. For example Irgarol concentration may have been influenced by variation in the intensity of boating activity between individual navigable broads. Similarly, hydrological variation between broads along the River Bure will have had an influence on the nature of their bed sediments, such as the slightly higher organic matter content found in the more downstream broads. Differences in the partitioning of organic biocides between water and sediment compartments between sample sites also plays a part in explaining variation in the observed triazine sediment concentrations.

The bottom sediments in shallow aquatic environments are susceptible to resuspension into the overlying water column, especially where boat movement and propellers cause additional disturbance. Therefore there may never be chemical equilibria of compounds partitioned between the bottom sediments and the overlying water, as physical mixing of the compartments alters the partitioning dynamics. However, some information on the environmental fate and partitioning of organic contaminants can be gained from analysis of their distribution between water and sediment compartments in the field. The solid-water partition coefficient K_d based on field data (Long *et al.* 1998;Bowman *et al.* 2003), provides a measure of the

environmental partitioning of a compound between, in this case, that adsorbed to the bottom sediments and that dissolved in the water column (see following equation).

$$K_d' (\text{l kg}^{-1}) = \frac{\text{concentration (ppb) in bottom sediments (µg kg}^{-1}\text{ DW)}}{\text{concentration (ppb) in aqueous phase (µg l}^{-1})}$$

The data presented has been analysed with a view to demonstrating the variation in partitioning of Irgarol and atrazine between water and sediment compartments within the River Bure freshwater system. The values generated are specific to the water chemistry and sediment character of each sample site, so there is some inherent variability in values between sample sites. Nonetheless, mean K_d' values obtained from the August 2004 biocide data demonstrate a significant difference in partitioning between the two triazine compounds. There is also a relatively large variation between sample sites in the calculated Irgarol K_d' values.

Table 4.2 Field partition coefficients K_d' (l kg^{-1}) of August 2004 sample sites.

K_d' (l kg^{-1})	Irgarol	Atrazine
Mean	126.7	19.5
S.D.	95.2	9.1
n	21	27

Higher K_d' values indicate a greater proportion of biocide associated with the sediment phase compared to the aqueous phase. This measure of K_d', however, does not represent the complete partitioning between solid and water, as the concentration of biocides associated with suspended sediments was not determined, as water samples were cellulose nitrate membrane filtered (0.45 µm) prior to solid phase extraction. The fraction of biocide adsorbed to organic carbon or colloidal matter < 0.45 µm, retained on the membrane filter, was also not quantified. The approach does however provide information on the relative distribution of these biocides, both between the two triazines and the sample sites in the study area. The results in Table 4.2 suggest that Irgarol was partitioned to a significantly greater extent than atrazine to the bed sediments along the River Bure waterway (Student unpaired t-test, $t = 5.84$, $p = <0.001$, $df = 46$). A Mackay fugacity modelling comparison of these two compounds also showed that Irgarol was more likely to

partition to the sediment phase than atrazine (Voulvoulis, Scrimshaw, and Lester 1999b). The greater hydrophobicity of Irgarol may account for some of the relative difference in K_d between the compounds. Konstantinou *et al.* (2004) suggest that Irgarol is the most hydrophobic of the triazine family of biocides due to it having both a tert-butyl and a cyclopropyl functional group. Experimentally derived log K_{ow} (octanol-water partition coefficient) values reported for Irgarol range from 2.8 – 4.1, whereas a lower range of 2.6 – 2.7 is reported for atrazine (see Appendix 9.1). These data support the observation of a greater proportion of Irgarol being associated with the River Bure bed sediment compared to atrazine. However given the spatial variability in sediment character and biocide concentration results, the precise nature of the partitioning i.e., the proportion of sorption to organic matter and clay particles, remains unclear.

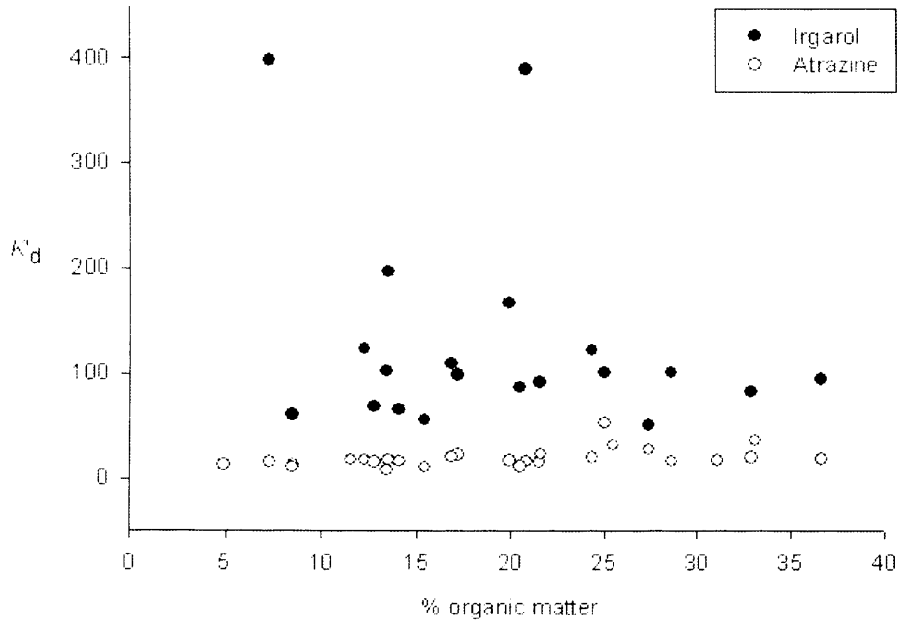

Figure 4.17 Scatterplot of percentage organic matter against field-based solid-water partition coefficient (K_d) for Irgarol and atrazine.

Figure 4.17 shows no obvious variation in either triazine K_d values across the range of sediment organic matter content sampled, as confirmed by subsequent correlation analysis. The relatively large variation observed in Irgarol K_d values (Table 4.2) can be seen to be influenced by the two sample sites with very large values close to 400. These sites were Landamores dyke, a boatyard and maintenance area, and Hudsons Bay, a connected broad.

In some of the sample sites, especially those exposed to intense boating activity and maintanence, antifoul paint flakes may be present in the sediments (Rogers, Watts, and Johnson 1996;Zhou *et al.* 1996), which would increase the distribution ratio between the two phases (Bowman *et al.* 2003). However there was no overall pattern that would suggest that more contaminated sites such as boatyards exhibited significantly higher K'_d values than the other site types sampled. A boatyard site, Landamores dyke, did have one of the highest K'_d values, but so did Hudson's Bay, which, whilst being positioned just downstream of Wroxham village, is not directly navigable. By comparison atrazine had a relatively small variation in the mean K'_d value from the same sample sites. This suggests that there were different factors determining the spatial distribution and partitioning behaviour of these two triazine compounds, but the factors determining relative Irgarol water-sediment distribution are not clear from these results.

Given the greater variability in K'_d values for Irgarol, other water quality parameters such as electrical conductivity and pH were considered as environmental factors with the potential to influence partitioning between the water and solid compartments. However from the K'_d values, no significant relationships were found within the dataset. A laboratory study specifically testing for variation in the partitioning behaviour of Irgarol also found no clear relationship with changes in pH or salinity (Voulvoulis *et al.* 2002).

4.3 Conclusions

The AFP biocides Irgarol and diuron were successfully quantified at low ng l^{-1} concentrations in water samples collected from the River Bure waterway. These positive detections were despite the sample collection occurring up to two years after these particular biocides were banned for use in antifoulant paints. Furthermore, the Irgarol concentration levels quantified in the present study were relatively high compared to values previously reported for freshwaters.

Variability through time and space of AFP biocide and agricultural herbicide concentrations reflected differences in their input to the environment, and to some extent the subtle differences in their physico-chemical properties. Dissolved Irgarol spatial distribution was found to be strongly associated with the level and type of boating activity at individual sample sites. Highest average concentrations were

observed in boatyards. Variation in concentration between boatyard sites was high and may have resulted from several site-specific factors. These potentially included the density of moored boats; the type and size of boats moored and whether they were treated with antifoul paint; water retention time in the boatyard basin; and whether re-painting and other maintenance activities took place at the site. No antifoul biocides were detected at sites isolated from navigation and there was no significant difference between Irgarol concentrations within naviagble and connected sites over the course of the study. Diuron in the aqueous phase was predominantly detected in boatyard sites, with none at all detected from isolated broads, connected broads or river channel sites.

The triazine biocides Irgarol and atrazine were also successfully quantified at low ng g^{-1} concentrations in sediment collected from the River Bure waterway. During August 2004 Irgarol was found in quantifiable concentrations at every sample site that was open, or hydrologically connected, to boating activity. There was close similarity in the spatial distribution pattern of Irgarol concentrations determined from both the aqueous and sediment phases across the study area. Variation in the physical characteristics and composition of bed sediments was not found to have any significant influence over the spatial pattern of Irgarol sediment concentrations from the data collected. The highest Irgarol sediment concentrations were again observed from within boatyard sites, notably Landamores dyke and Horning Ferry Marina. Heavy Irgarol contamination at such concentration "hotspots" may disrupt model predictions of the partition distribution of biocide between dissolved and sediment phases, through the presence of AFP biocide containing paint flakes (unquantified in the present study) which may act to masked predictions based on partitioning to organic matter.

During the collection of surface sediments, coloured red and blue paint flakes were visible within the sediment at some boatyard sites, particularly the two most contaminated. Boat maintenance involving scrubbing, sanding and pressure hosing of antifoul painted hulls prior to re-painting generates such flakes. If these activities are carried out near the waters edge and sufficient precautionary steps are not in place to prevent environmental contamination, flakes can become washed into the waterway and thus become incorporated into the sediments. Further work on the influence of biocide paint flakes is needed to assess their effects on partitioning and biocide persistence in freshwater systems.

Atrazine has a widespread and diffuse source from agricultural applications, with the data showing that its spatial distribution was not related to boating activity as it was detected at all of the sites sampled. Atrazine presence at sample points in the river headwaters, and also isolated lake sites with small catchment areas, shows that it is a common aquatic contaminant within the Norfolk landscape. Greatest dissolved atrazine concentrations occurred in the river channel sites and navigable broads. Determination of atrazine concentrations in the present study was highly valuable for elucidating the processes influencing the spatial distribution patterns of the structurally analagous Irgarol compound. The sediment-water distribution coefficient (K_d) for both triazine biocides studied was not found to be influenced by sediment organic matter when analysed at the catchment scale. It was the degree of connectivity to the main river channel that was most closely associated with atrazine concentrations, whereas the type and level of boating activity at sample sites was most closely associated with Irgarol contamination.

This sampling regime, set at the catchment scale, clearly showed that Irgarol and diuron contamination in the River Bure waterway was derived from boating activity. This is supported by the observed seasonal variation in water concentrations; through spatial analysis; and analysis of confounding environmental factors at each sample site. A gradient of AFP biocide contamination was determined between different site types, with the greatest concentrations occurring in boatyards, with lower contamination in navigable and connected sites, and finally none at isolated sites. Through quantification of contemporary biocides in water and sediment compartments, an understanding of the inputs and environmental fate of boat-derived contaminants within the River Bure waterway has been gained. This information is not only valuable in itself, but will assist interpretation of the observed contamination of TBT in the surface sediments of the River Bure waterway as detailed in Chapter 5.

CHAPTER 5 – ORGANOTIN SURFACE SEDIMENT CONTAMINATION

5.1 Introduction

Tributyltin contamination of freshwater environmental media has been documented for a number of lakes and rivers exposed to recreational boating and commercial shipping activity (Chau 1986;Waite *et al.* 1989;Stang and Goldberg 1989;Becker van Slooten and Tarradellas 1995;Fent and Hunn 1995). The distribution of TBT contamination has been shown to be positively related to the degree of exposure to boating activity across a range of spatial scales, from individual river systems to wider regional zones (Dowson *et al.* 1992;Gomez-Ariza *et al.* 1998). Locations such as marinas, boatyards and other boat maintenance facilities have been identified as having greater TBT surface sediment concentrations than in areas of lower boating activity, due to the greater exposure from antifoulant paints (Maguire *et al.* 1982;Schebek *et al.* 1991;Dowson, Bubb, and Lester 1993c).

Much of the baseline environmental research on TBT contamination was conducted in the late 1980s and early 1990s when TBT was banned in many regions of the world for use as antifoulant agent on small vessels <25 m. The restrictions on TBT usage resulted in the successful reduction in dissolved TBT concentrations (Dowson *et al.* 1994), but concentrations within sediments have been shown to have declined at a lesser rate (Dowson *et al.* 1993c). Degradation of TBT in sediments is slow, with an experimental study measuring TBT breakdown in anoxic freshwater sediments indicating that the half-life of TBT under these conditions was in the order of decades (Dowson *et al.* 1993c). Sediment core studies have shown that the environmental persistence of TBT is such that clear pollution profiles can be determined with sediment depth (Page *et al.* 1996;Wade *et al.* 2004;Almeida *et al.* 2004;Scrimshaw *et al.* 2005;Sayer *et al.* 2006). Detectable concentrations therefore remain in surface sediments in many previously contaminated areas dispite over a decade of total or significantly reduced TBT inputs. Generally, lower TBT and DBT concentrations have been found in surface sediments compared to deeper layers due mainly to successful legislative reductions in usage, but also through loss to overlying water through diffusive flux (Burton, Phillips, and Hawker 2005).

The presence of areas of intense TBT environmental contamination was also found to occur along the waterways of the River Bure, Norfolk, UK (Waite *et al.* 1989).

Given the history of intense boating activity and usage of TBT in this inland waterway (MAFF 1993), this chapter aims to determine whether a contamination gradient exists in the contemporary surface sediments; what the similarities are to the organic AFP biocide contamination gradient detailed in Chapter 4?; what is the current distribution of AFP biocides, including TBT, in a non-navigable, but hydrologically connected broad?; and determine the temporal patterns of organotin contamination from sediment cores collected in the study area.

5.2 Results and discussion

5.2.1 Analytical quality control

QA/QC procedures were followed as outlined in (Waldock and Waite 1994). No analytical blank had any quantifiable organotin. Residual standard deviation (RSD) of the Response Factor sample peak heights were < 10% for MBT, DBT and TBT for all sample runs (minimum n = 6). RSD for triphenyltin (18.3%) was greater than for the butyltins. Triplicate analysis of environmental samples from Landamores boatyard and Ranworth Broad produced RSD results given in Table 5.1 below.

Table 5.1 Peak height %RSD of extracted organotins for Response Factor and environmental samples.

	Organotin species % RSD			
	TBT	DBT	MBT	
Response Factor RSD range	1.7 – 6.3	1.1 – 4.7	1.5[a]	
Landamores boatyard	97	85	41	n = 3
Ranworth Broad	7.0	5.2	nd[b]	n = 3

a – based on one run for MBT analysis, n = 7
b - not detected

5.2.2 Sediment organotin concentrations

Of the 15 surface sediment samples taken in April 2003, 12 had quantifiable concentrations of TBT (see the top half of Table 2.1 for a description of these sample sites and Figure 2.4 for location map). Quantifiable concentrations ranged from 34 - 3319 ng g^{-1}, with a mean concentration of 811 ng g^{-1} (± 1203). The sites with no detectable TBT were u/s Horstead Mill and Cockshoot Broad, which are both isolated from navigation, and Hoveton Great Broad, which has a surface water

connection to the river, but no access to navigation. These three sites also had no quantifiable DBT. The other connected broad sampled in April 2003, Ranworth Broad, gave quantifiable TBT and DBT concentrations, despite not being directly open to navigation. At the most upstream navigable site sampled, u/s Wroxham Rail Bridge, the lowest positive TBT concentration was recorded of the 15 sites, at 34 ng g^{-1}. At this site DBT was < LOQ. All navigable sites had quantifiable TBT concentrations. The greatest TBT concentrations were from the five boatyard sample sites. By far the highest concentrations were from Horning Ferry Marina (3319 ng g^{-1}) and Landamores Dyke (3288 ng g^{-1}).

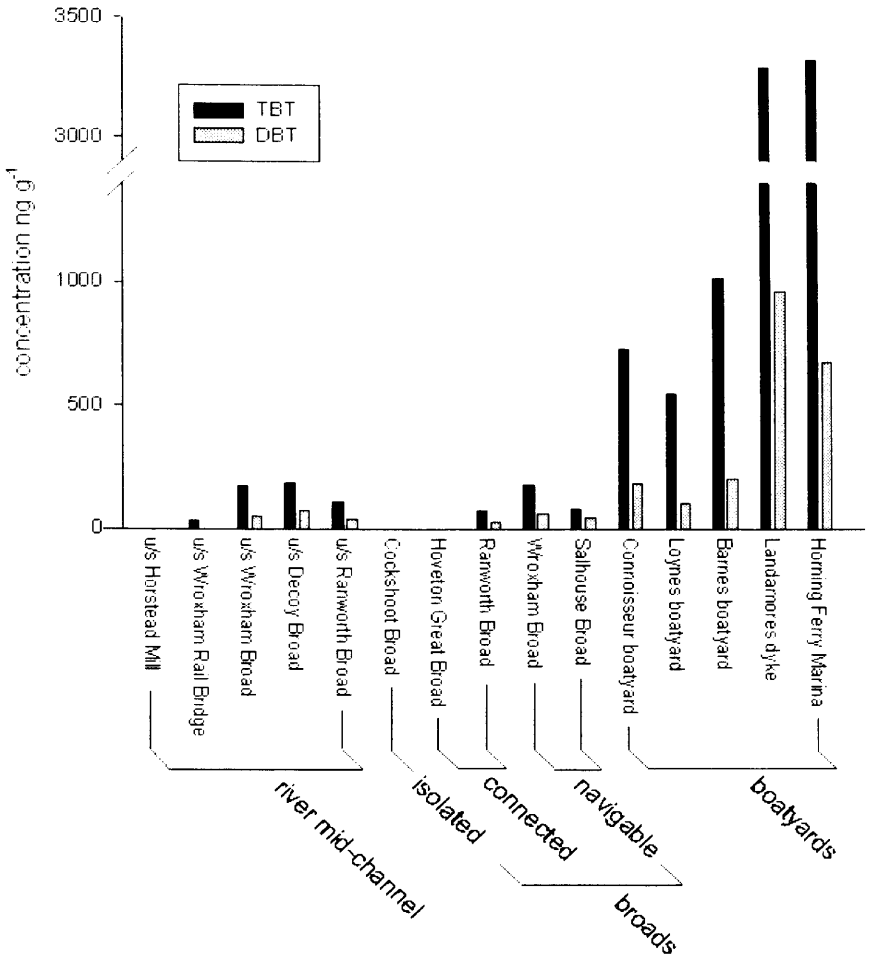

Figure 5.1 Surface sediment butyltin concentrations, April 2003.

The boatyard sites also gave the highest DBT concentrations of sites sampled in April 2003. The range of quantifiable DBT concentrations from all sites was 29 to 963 ng g^{-1}, with a mean concentration of 220 ng g^{-1} (± 308). MBT was not analysed

from these sediment samples. No triphenyltin was detected in any of the April 2003 sediment samples.

The original 15 sites were sampled again in August 2004 along with 17 other sites within the River Bure catchment area, to give a wider range of samples from areas of differing boat activity (see bottom half of Table 2.1 for site descriptions). In addition, the monobutylated organotin species, MBT, was also determined from this set of surface sediment samples. Of the 32 sites sampled 17 (53%) had quantifiable TBT concentrations in the range 34 - 6890 ng g^{-1}. Quantifiable concentrations of DBT and MBT ranged from 22 - 965 ng g^{-1} and 126 - 614 ng g^{-1} respectively (Figure 5.2). No butyltin species was detected in samples from any of the sites isolated from navigation, or those downstream of WWTW discharges. No butyltins were detected at the two most upstream navigable sites, d/s Belaugh WWTW and u/s Wroxham Rail Bridge. In the connected broads, Salhouse Little, Decoy and Ranworth Broads had quantifiable organotins. The connected sites where no organotin concentrations were quantifiable were Hoveton Great Broad and the adjoining Hudson's Bay. Butyltin concentrations were quantifiable from the navigable broads, with the exception of Hoveton Little Broad. This site has historically been closed to navigation for the winter, from November through to the end of April, so has had reduced exposure to boats coated with TBT antifoul paints compared to other broads open to year-round navigation. Hoveton Little Broad was also suction dredged in 1990 (George 1992), shortly after the withdrawal of TBT use in the broads. Sediment bound TBT contamination present within the site may therefore have been reduced through this operation. Of the navigable broads, the two most downstream broads sampled, Malthouse and South Walsham, both had greater organotin concentrations than the other three. Concentrations at these two sites were similar to the least contaminated boatyards. Between the sample site types, on average, TBT and DBT were detected at the greatest concentrations in the boatyard/marina sites. Triphenyltin was detected at Barnes boatyard and u/s South Walsham Broad. This compound has had applications as an AFP and as an agricultural pesticide (Stab *et al.* 1994;Eng *et al.* 2002). Peak heights for triphenyltin on the chromatogram were too small to be quantifiable, and further work is required to unequivocally confirm the presence of this compound in the River Bure waterway.

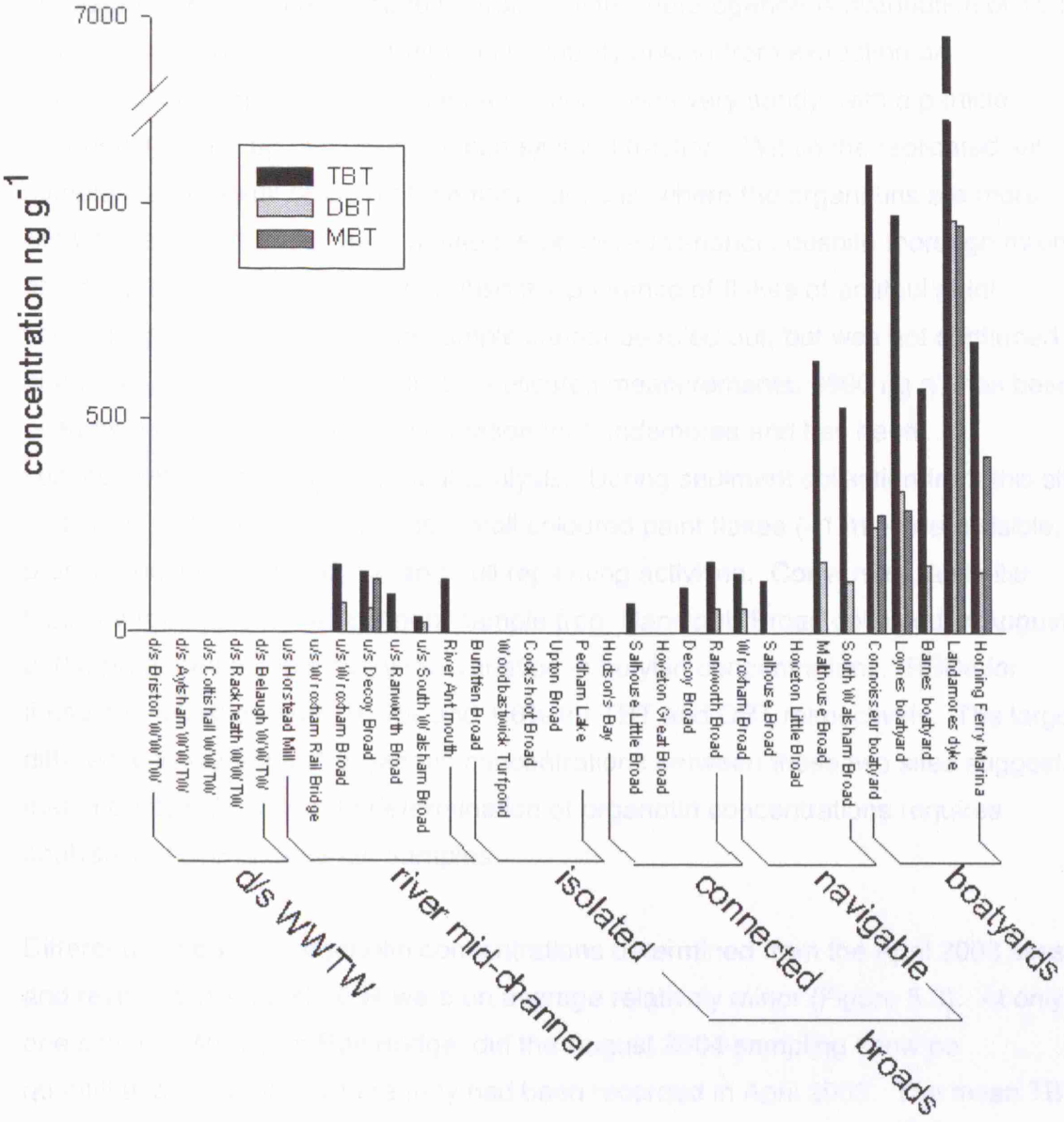

Figure 5.2 Organotin sediment concentrations, August 2004.

The sediment sample taken from Landamores in August 2004 was thoroughly homogenized and divided into three 1 g sub-samples for separate extraction and analysis. The TBT concentrations of each replicate were 979, 4536 and 15155 ng g^{-1}, which gave an RSD of 97% (Table 5.1). Similar high variance in concentration was observed for DBT and MBT, with RSDs of 85% and 41% respectively. This variance within a single sediment sample is much higher than the analytical RSD determined from the Response Factor samples, which was always < 7%. The RSD of TBT concentrations determined from spiked reference sediment

(BRC-646) was 5.1%. The observed difference between sub-samples from the Landamores site is presumed to be from a highly heterogeneous distribution of TBT within the sediment matrix rather than variability arising from extraction or quantification. The sediment from Landamores was very sandy, with a particle distribution made up of 67% in the coarse sand fraction. Within the replicated sub-samples an unequal division of the finer fractions, where the organotins are more likely to be bound, may have caused the observed variance, despite thorough mixing and accurately weighed portions. Also the presence of flakes of antifoul paint containing TBT in the sediment sample cannot be ruled out, but was not confirmed analytically. The mean of the three replicated measurements, 6890 ng g^{-1} has been used as the recorded TBT concentration for Landamores and has been subsequently used in all statistical analysis. During sediment collection from this site and some of the other boatyards, small coloured paint flakes (<1 mm) were visible, presumably from scrub down and hull repainting activities. Conversely, a similar triplicate replication of a sediment sample from Ranworth Broad collected in August 2004, produced comparitively low variation in butyltin concentrations. RSDs for these sub-samples were 10.7% and 1.6% for TBT and DBT respectively. The large difference in variance of organotin concentrations between these two sites suggests that in boatyards, accurate determination of organotin concentrations requires analysis of several separate samples.

Differences between organotin concentrations determined from the April 2003 sites and revisited in August 2004 were on average relatively minor (Figure 5.3). At only one site, u/s Wroxham Rail Bridge, did the August 2004 sampling show no quantifiable organotins where they had been recorded in April 2003. The mean TBT concentration for the original 15 sampled sites sampled again in August 2004 was 1001 ng g^{-1} (± 1987), an apparent increase of 190 ng g^{-1}. There were two sites however that showed marked variation in TBT concentration between the two sampling occasions. The mean TBT concentration at Landamores in August 2004 (6890 ng g^{-1}) was much higher than in April 2003 (3288 ng g^{-1}). The August 2004 value was increased due to the one very high value determined from one of the replicated sub-samples, as already discussed. The other site with a large difference in butyltin concentrations between the two sampling periods was Horning Ferry Marina. At this site there was an apparent decrease in TBT concentration between April 2003 and August 2004, from 3319 ng g^{-1} to 680 ng g^{-1} respectively, however the significance of this apparent trend is unknown, as replicate analyses of sub-samples

was not conducted on these samples. No such decrease in TBT concentrations was observed at any other site, so a heterogeneous distribution of TBT within the sediment is suggested as the cause of this variation, rather than degradative processes.

Similar to Landamore's, the sediment at the Horning Ferry marina sample site had a relatively high content of coarse sand, 69 %. The high sand content may be explained by construction and dredging activities altering the substrate from that naturally found in the area. Sand and gravel back-fill was certainly used along the quay headings in the Horning Ferry Marina, and may have been incorporated behind similar structures along Landamores Dyke.

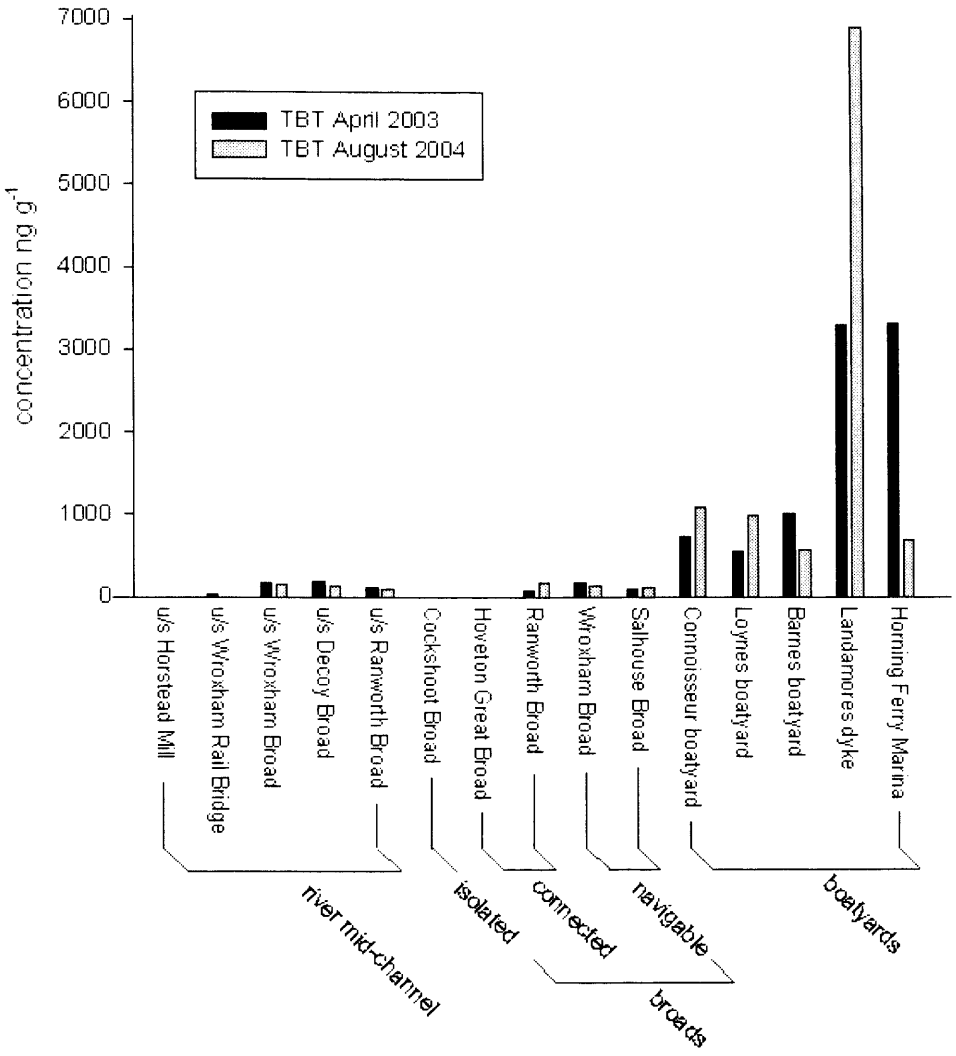

Figure 5.3 Comparison of TBT concentrations between sites sampled April 2003 and August 2004.

Further research is required to determine whether it is the high sand content that makes representative sub-sampling of boatyard sediments difficult, or whether it is paint flakes at these sites, that gives rise to such high variance within sediment samples collected on different dates.

Comparison of all the butyltin data from the different site types sampled shows that the boatyard sites had the highest mean concentrations for all analysed butyltin species. The next highest mean concentration was within the navigable sites, followed by the connected sites. MBT was not detectable in any of the connected broad sample sites. No butyltin species were detected in the sites completely isolated from navigation. The spatial pattern of TBT contamination, as determined by this study, shows that a gradient in concentration exists between sites with differing exposure to boating activities. The gradient is still measurable despite there being more than a 16 year period between the sampling programme undertaken in the present study and the 1987 ban on TBT usage in antifoul paint on boats < 25 m in length. These patterns are also observable despite considerable maintanence dredging activity, especially in the boatyard areas, which will have disturbed and redistrubuted contaminated sediments. This sampling has revealed no other significant source of butyltins to the River Bure sediments other than the historical deposition from antifoul paints applied up until 1987.

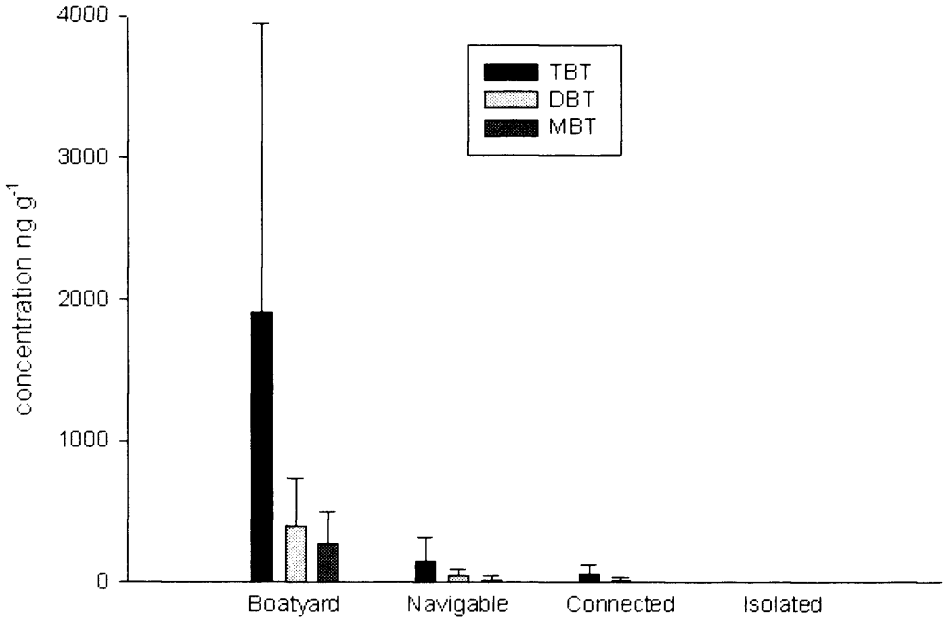

Figure 5.4 Mean butyltin species sediment concentration by site type (± 1 S.D.). (April 2003 & August 2004 data).

To test whether the TBT data for each site type was statistically different at a confidence interval of >95%, the non-normal, unequal variance data were analysed by the non-parametric Mann Whitney method (with adjustments for ties). Within the data there was sufficient evidence to conclude a significant difference in TBT concentrations between boatyard sites and navigable sites (W = 233, p = <0.001) and also between boatyards and connected sites (W = 125, p = <0.001). However between the navigable and connected sites there was insufficient evidence to conclude that a significant difference existed between the median TBT values, W = 263, p = 0.09.

Highly significant correlations between log transformed TBT and DBT sediment concentrations from both the April 2003 (r = 0.982, p = <0.001, n = 12) and August 2004 (r = 0.988, p = <0.001, n = 13) datasets were found to exist, see Figure 5.5. This observation is highly supportive of the presumption that the presence of DBT in the sediments of the River Bure waterway is predominantly derived from the breakdown of TBT.

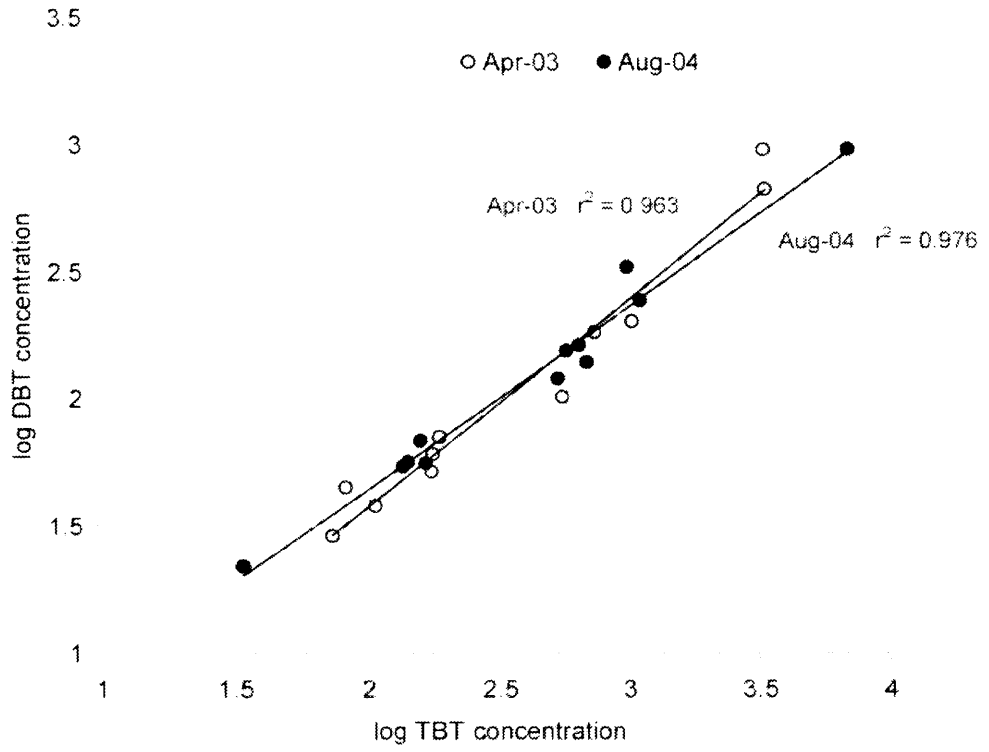

Figure 5.5 Relationship between TBT and DBT sediment concentrations.

Within the August 2004 data, no significant relationships between sites with quantifiable sediment TBT concentrations and sediment characteristics of percentage organic matter, clay, silt, fine sand or coarse sand were found.

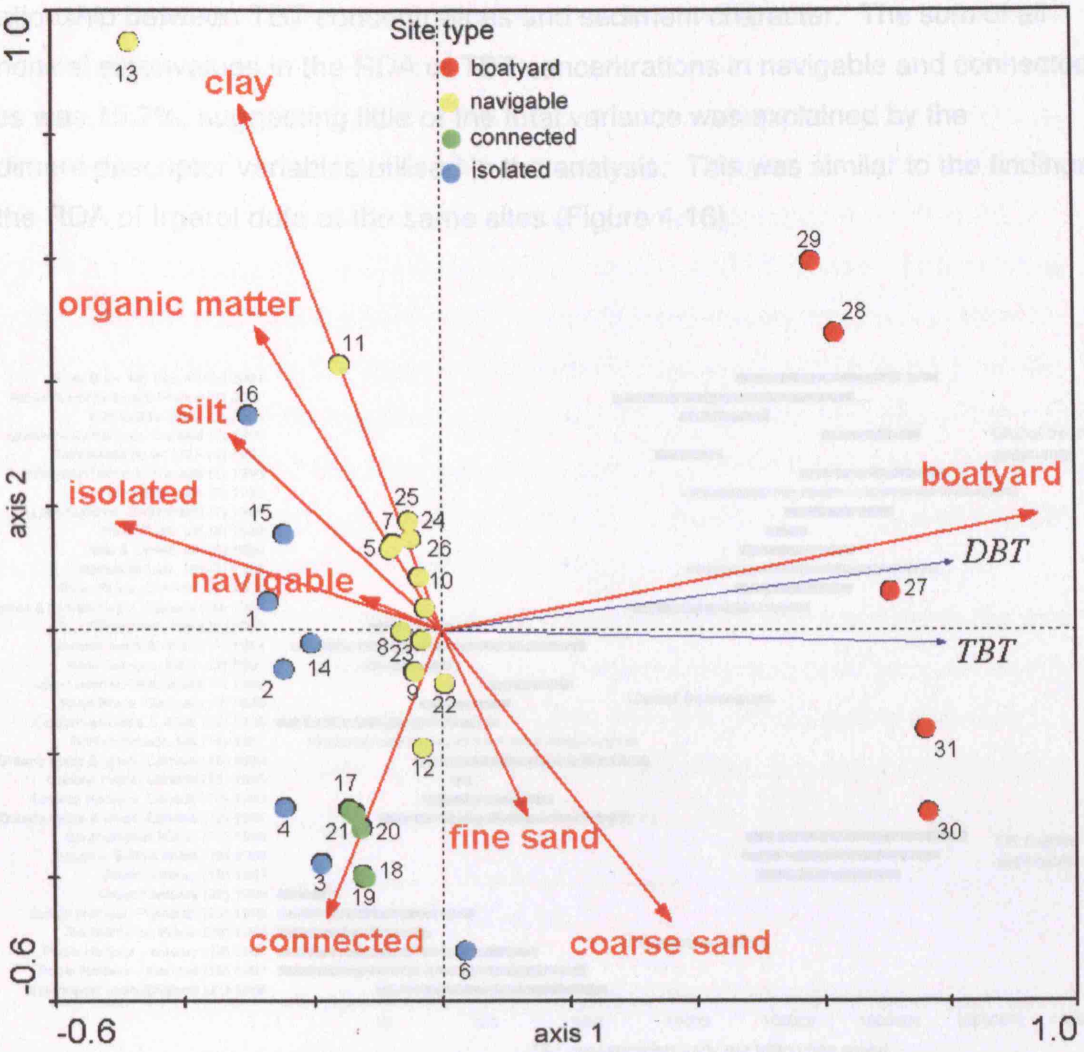

Figure 5.6 RDA triplot of butyltin concentration with sediment character and site type.

Redundancy analysis (RDA), a direct gradient analysis, showed that site type was the descriptor variable that explained most of the variation in the TBT concentration data, with sediment physical characteristics explaining relatively little of the variance (Figure 5.6). Left to right (axis 1) across Figure 5.6 represents a gradient of increasing TBT contamination and also a site type series, which goes from isolated, connected, navigable and through to boatyards on the far right (red circles). The

spatial distribution of TBT, as determined in this study, was therefore most influenced by the type of sample site where the sediments were collected from. Axis 1 was found to explain 67.8% of the variation in the dataset.

Removal of boatyard and isolated sites from this analysis did not reveal any close relationship between TBT concentrations and sediment character. The sum of all canonical eigenvalues in the RDA of TBT concentrations in navigable and connected sites was 15.7%, suggesting little of the total variance was explained by the sediment descriptor variables utilised in the analysis. This was similar to the findings in the RDA of Irgarol data at the same sites (Figure 4.16).

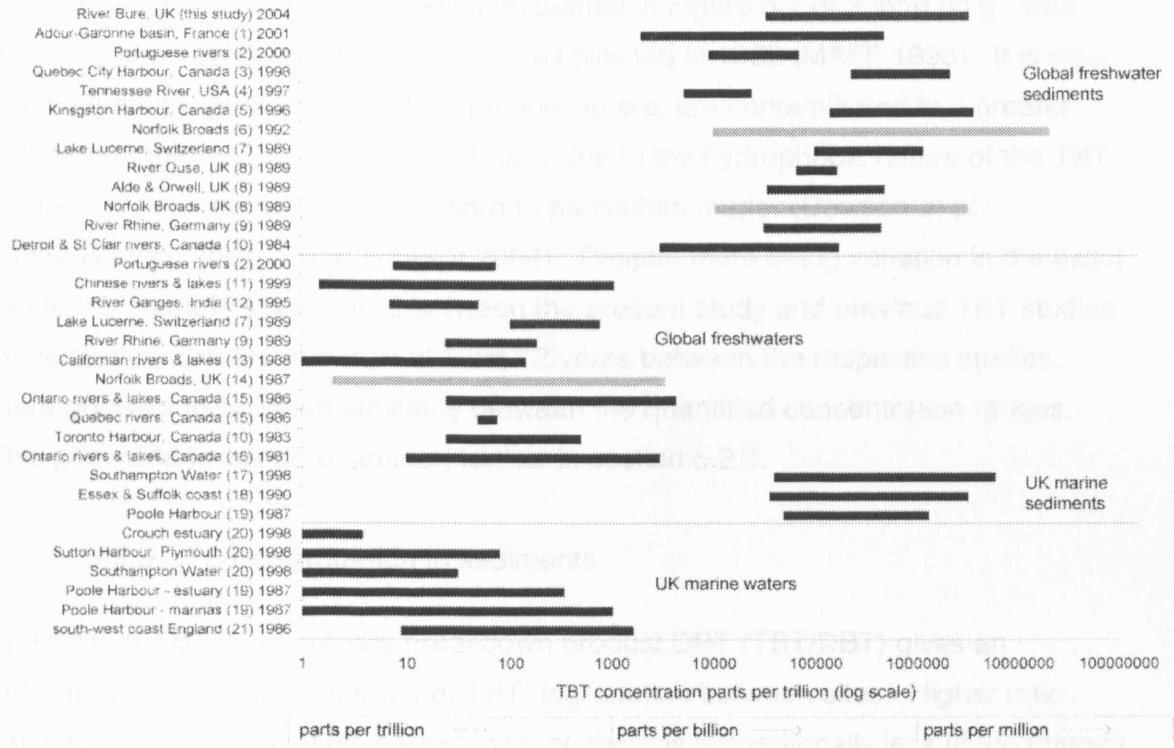

Figure 5.7 TBT concentration ranges reported in the literature (blue bar = the present study, grey bars = other Broads studies)

References: 1 - (Bancon-Montigny, Lespes, and Potin-Gautier 2004), 2 - (Diez *et al.* 2005), 3 - (Regoli *et al.* 2001), 4 - (Loganathan *et al.* 1999), 5 - (Chau *et al.* 1997), 6 - (MAFF 1993), 7 - (Fent *et al.* 1991), 8 - (Dowson *et al.* 1992), 9 - (Schebek *et al.* 1991), 10 - (Maguire *et al.* 1985), 11 - (Jiang *et al.* 2001), 12 - (Ansari *et al.* 1998), 13 - (Stang and Goldberg 1989), 14 - (Waite *et al.* 1989), 15 - (Maguire and Tkacz 1987), 16 - (Maguire *et al.* 1982), 17 - (Thomas *et al.* 2000), 18 - (Dowson *et al.* 1993c), 19 - (Langston 1987), 20 - (Thomas *et al.* 2001), 21 - (Cleary and Stebbing 1987a).

The level of TBT quanitifed in this study is comparable to concentrations reported for contaminated freshwater sediments from around the globe (Figure 5.7). The numbers of studies reporting TBT contamination in the marine environment is far greater than in freshwaters, so to simplify comparison of concentrations reported from marine and freshwaters, only marine concentrations from UK based studies are shown.

The top bar represents the range of quantifiable TBT concentrations determined in the present study. The grey bars represent the results of previous TBT studies conducted in the Norfolk Broads. Results from the present study are within the ranges of both studies that previously reported sediment TBT concentrations in the Broads (Dowson *et al.* 1992;MAFF 1993). The highest freshwater TBT sediment concentration reported in the studies presented in Figure 5.7 of 20650 ng g^{-1} was from Landamores dyke on the River Bure collected in 1992 (MAFF 1993). It is clear that sediments, whether in fresh or marine waters, are contaminated to a greater extent than their overlying waters. This is due to the hydrophobic nature of the TBT compound, and its high affinity to sorb to particulate matter (Dowson *et al.* 1993a;Burton, Phillips, and Hawker 2004). Despite there being variation in the exact location of sample sites visited between the present study and previous TBT studies in the Norfolk Broads and also at least 12 years between the respective studies, there appears to be close similarity between the quantified concentration ranges. This phenomena will be examined further in section 5.2.3.

5.2.3 TBT degradation in sediments

The ratio of TBT to its primary breakdown product DBT (TBT/DBT) gives an indication of the relative amount of TBT degradation between sites. Higher ratio values suggest greater TBT persistence, as there is proportionally less of the primary breakdown product DBT. As no alternative detectable inputs of DBT or MBT could be determined from the sampling programme adopted, concentrations of these species are predicted to have derived from the degradation of TBT leached from antifoul paints. All boatyard sites had simultaneously detectable TBT and DBT concentrations, whereas only two-thirds of the navigable sites sampled had simultaneously detectable concentrations. As MBT was not analysed for from all samples collected, DBT concentrations alone have therefore been used to determine the relative degradation of the parent TBT species between sites. During August

2004 when MBT samples were analysed for, only one site outside of boatyards was found to have a quantifiable concentration, so inclusion of MBT would have added little information to the wider comparison of degradation of TBT at sites within the study area (Table 9.15 in Appendix 9.4 for MBT concentrations).

The highest TBT/DBT ratio values came from boatyards with a maximum of 7.1 from the Landamores dyke sample taken in August 2004. The river mid-channel site, u/s South Walsham Broad, sampled in August 2004 had the lowest value of 1.6. Comparison of the TBT:DBT ratios from the August 2004 sampling were similar to those determined from the April 2003 samples. South Walsham Broad and Malthouse Broad had particularly high ratio values compared to the other navigable broads sampled, with calculated ratios of 4.4 and 3.9 respectively. These two sites also had the greatest TBT concentrations of the navigable broads. The pattern of sites with high TBT concentrations also exhibiting high TBT/DBT ratios is suggestive of a link between the level of TBT contamination and its persistence through time, ie. greater environmental persistence at sites with higher contamination.

Data from both sampling occasions, giving a total of 10 boatyard samples gave a mean TBT/DBT ratio of 4.6 ± 1.2. The mean value for the 11 samples from navigable sites was 2.8 ± 0.8. Only two samples from connected sites had simultaneously detectable TBT and DBT and in both cases the TBT:DBT ratio was < 3.

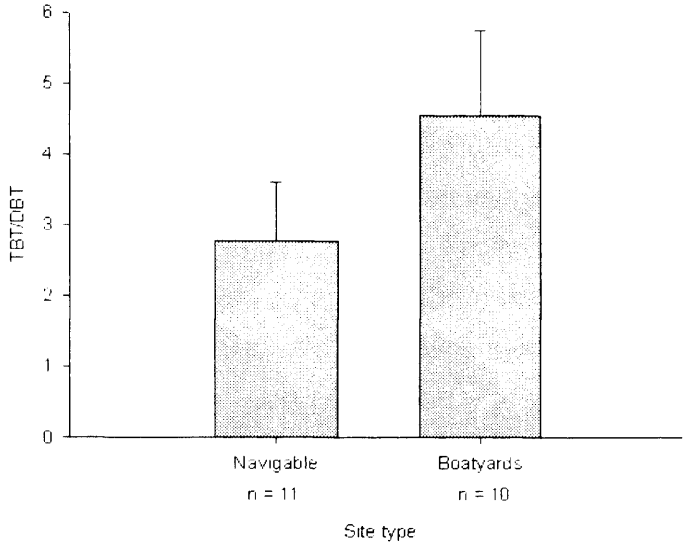

Figure 5.8 Mean TBT/DBT ratios for navigable and boatyards sites (± 1 S.D.).

The mean TBT/DBT ratio values for the boatyard samples were found in a 2-sample T-test to be significantly higher then those from naviagble sites (T = -0.401, p = <0.001). This data suggests that TBT degradation in surface sediments is greater outside of boatyards, or another way to look at the same pattern, is that TBT preservation in boatyard sediments is somehow greater. Analysis of Broads butyltin data from surface river sediments sampled from navigable areas in 1989 (Dowson *et al.* 1992) gave a range of 0.9 - 8.6 with a mean value of 2.7 ± 2.3 (n = 9). This previous study shows a similar range and mean of TBT/DBT ratio values to the navigable sites sampled in the present study. There were too few samples collected from boatyards to allow meaningful comparison. From these small datasets, the proportion of TBT and DBT in surface sediments appears to have changed comparatively little over time. A tentative conclusion is that the rate of TBT degradation to DBT in surface sediments has remained roughly constant.

In the present study, no sites had quantifiable DBT concentrations without there being TBT present. Similarly no site had MBT present without TBT and DBT also being present. This data supports the mechanism of stepwise debutylation of TBT in the Broads aquatic environment, as established in previous studies (Maguire and Tkacz 1985;Cleary and Stebbing 1987a). Identification of DBT and MBT emanating from sources other than degradation of the parent TBT compound have not been identified through the spatial sampling programme adopted in the present study. This further suggests that contamination from TBT containing antifoul paints has been by far the predominant source of butyltin species to this river system.

TBT results determined from the River Bure in the present study (2003 – 2004) and previously reported concentrations along the same stretch of river (1986 – 1992) (MAFF 1993;Dowson *et al.* 1994), shows the variation in concentration between the two time periods (see Figures 5.9 to 5.11). For ease of comparison it has proven useful to separate TBT values determined from non-boatyard and boatyard sites, as TBT concentrations within boatyard sediments were consistently higher. The mean TBT concentration from boatyard sites in the present study was 1911 ± 2043, whilst the mean from the 1986 - 1992 period was 6026 ± 7464. The mean values indicate a reduction in surface TBT concentration over time and the clustering of relatively low TBT concentrations in the present study shows that most boatyard surface sediments are now less contaminated (Figure 5.9). However there are some boatyard sites sampled in 2003 - 2004 with relatively high TBT concentrations

(> 2000 ng g^{-1}), which is within the range of concentrations sampled around the time of the ban. The significance of this pattern is not clear, as there are too few data, but the dotplot clearly shows how two of the more recent boatyard samples have a similar concentration range to the historical data.

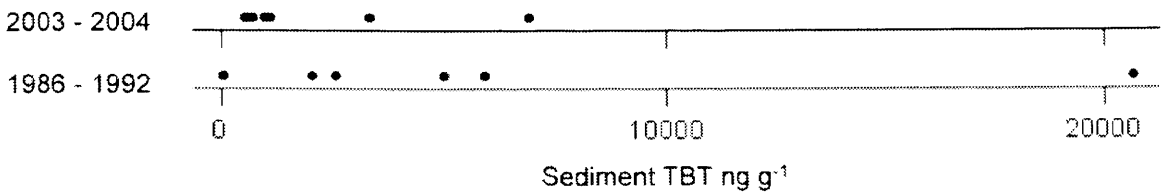

Figure 5.9 Dotplot of TBT concentrations sampled in River Bure boatyards at different time periods.
(2003 – 2004, n = 10; 1986 – 1992, n = 6).

The number of positive TBT concentrations reported from sites sampled outside of boatyard areas is more numerous both in this study and from the literature. This has enabled more accurate comparison of mean values than in the limited data collected from within boatyard sites. Additional surface sediment TBT concentrations were determined from two River Bure sites in 2000 (Sayer, unpublished data), which have been included within the more recent dataset with results from the present study. The 2000 - 2004 mean TBT concentration was 129 ± 147, whilst the 1986 - 1992 mean was 82 ± 134. The apparent increase in TBT concentration over time, outside of boatyards, may be an artefact of the increased sampling intensity within the more recent dataset. For example, both of the sites that had the highest TBT concentrations in the 2004 sampling, Malthouse Broad and South Walsham Broad (represented by the two outlier points in the right hand bar of Figure 5.10), had never been previously sampled for TBT. These findings are in sharp contrast to the results of Dowson *et al* (1994) that showed a 90 – 100% reduction in sediment TBT concentration between Broads sites sampled in 1989 and again in 1992. This reduction was attributed to the success of the 1987 retail ban on TBT in AFPs. However, given the similar inter-quartile ranges displayed for both time periods (as given in Figure 5.10), it would appear that, at the catchment scale, surface sediment TBT concentrations outside of boatyards have remained relatively constant since the ban on TBT usage in antifoul paints in the Broads.

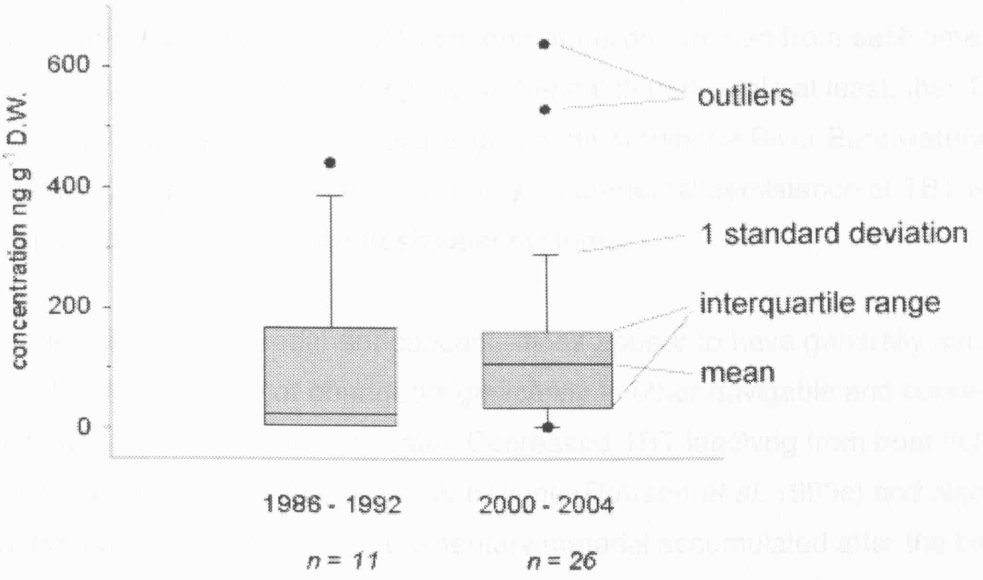

Figure 5.10 Boxplot of non-boatyard TBT concentrations from navigable and connected sites for two different time periods.

Presentation of the same data as dotplots reveals the distribution of the measured TBT concentrations. TBT values < LOD were substituted for LOD/2 within these navigable and connected sample sites, hence both datasets show a high frequency of samples near to zero (Figure 5.11). These data show that a greater frequency of concentrations between 50 – 200 ng g-1 has been determined in the more recent work, albeit through a greater sampling intensity. The more recent work does not however show an increased frequency of samples with TBT concentrations < LOD, as may be expected from an increased sampling intensity. These more abundant mid-range TBT concentrations determined between 2000 – 2004 are however within the range previously reported (MAFF 1993;Dowson *et al.* 1994).

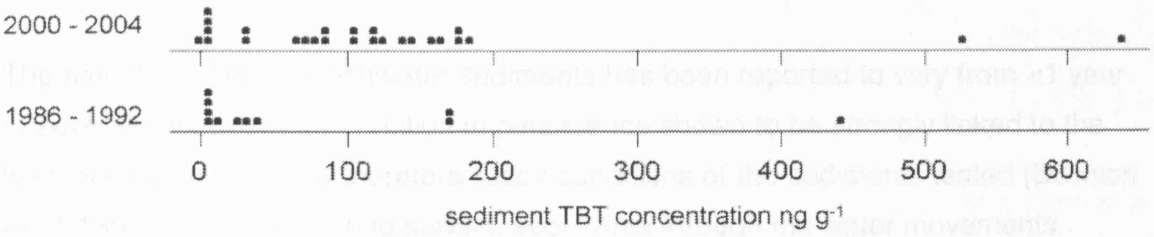

Figure 5.11 Dotplot of TBT concentrations from navigable and connected sites outside of boatyards.

A Mann-Whitney test revealed that there was not a significant difference between the respective median values of TBT concentrations determined from each time period ($W = 168$, $p = >0.05$), suggesting that at the catchment scale at least, that TBT concentrations have not decreased significantly within the River Bure waterway. This finding is significant in terms of the environmental persistence of TBT and its transportation within lowland freshwater systems.

Overall, TBT surface sediment concentrations appear to have generally decreased inside boatyards and not changed significantly in other navigable and connected areas at the wider catchment scale. Decreased TBT leaching from boat hulls has effectively reduced dissolved concentrations (Dowson *et al.* 1993c) and also the subsequent contamination of sedimentary material accumulated after the ban. This process has led to modern surface sediments in boatyards being on average less contaminated than previously reported. Sediment removal, degradation, remobilization and flushing will have all have contributed to the net loss of TBT from these heavily contaminated "hotspots".

Transportation of sediment contaminated with TBT is one explanation for the increased detection of mid-range ($50 - 200$ ng g^{-1}) TBT concentrations outside of boatyards. It is likely that there was a period of residual usage of antifoul paints containing TBT in the Broads for a short period after the ban on all craft <25 m, as boats coated prior to the ban would have a few years of use before re-application of replacement AFP. This gradual decrease in the total amount of TBT released from boats does not however account for the environmental persistence observed between the two datasets. Various studies have shown that decreased dissolved TBT concentrations occurred within popular boating areas after cessation of usage in antifoul paints (Valkirs *et al.* 1991;Cleary 1991).

The half-life of TBT in freshwater sediments has been reported to vary from <1 year to several decades, with variation in persistence shown to be strongly linked to the level of oxygenation and therefore redox conditions of the sediments tested (Dowson *et al.* 1993c). Disturbance to surface sediments through the water movements caused by boat propellers and to buried anoxic sediments from dredging activities represent mechanisms by which TBT contaminated sediment buried at depth in anoxic sediments can be mixed and returned to the surface in a relatively undegraded form. Given the heavy boat traffic and tidal rise and fall of the River

Bure, transport of TBT contaminated sediments away from hotspot areas is likely, though no direct evidence for this process occurring from boatyards is available. The following section investigates the mixing of organic AFP biocides, as well as TBT and its derivatives, within a single non-navigable broad.

5.3 Ranworth Broad spatial study

Irgarol was detected in water samples from the transects taken across Ranworth Broad in the range 3.8 - 5.5 ng l^{-1} (mean 4.8 ± 0.5). Analysis of sample number 10 failed due to an autosampler programming error, so no concentrations for any biocide were obtained from this water sample. Diuron was not detected in any of the transect water samples. Atrazine was detected from all points, with values in the range 27.6 - 34.3 ng l^{-1} (mean 32.2 ± 2.1). Isoproturon was not detected from any of the transect points, or indeed any of the other spatial survey sample sites along the River Bure during August 2004.

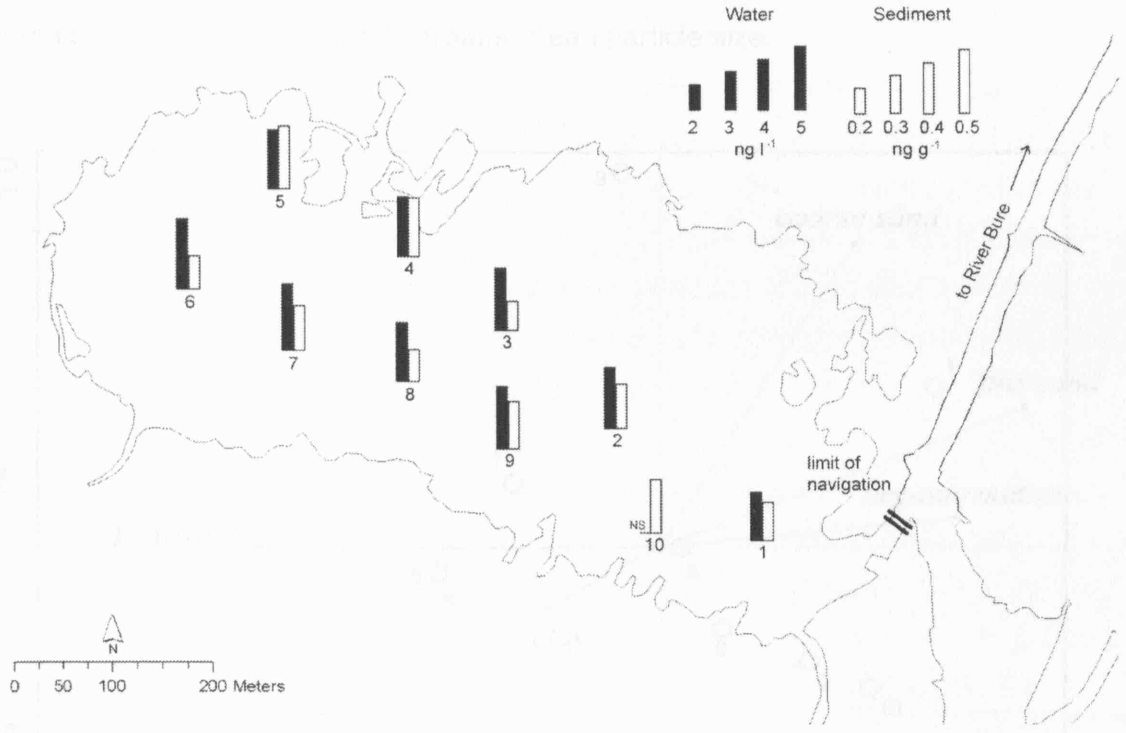

Figure 5.12 Irgarol concentrations from water and sediments in Ranworth Broad.

Irgarol sediment concentrations were quantifiable at all sample points in the range 0.15 - 0.40 ng g^{-1} (mean 0.27 ± 0.04). Atrazine was also detected in all sediment

samples, ranging from 0.31 - 0.44 ng g^{-1} (mean 0.37 ± 0.05). Isoproturon and diuron were not detected in any of the sediment samples.

There was only a small variance in the ranges of the biocide concentrations and also in the measured environmental variables. Therefore no significant relationships were found between biocide concentration in water or sediment with distance from the inlet or any of the water quality or sediment characteristics measured. Relationships were however found between the sediment physical characteristics and the distance of each sample point from the inlet. The sample sites closest to the inlet had a significant negative relationship with percentage organic matter ($r = -0.733$, $p = 0.016$, $n = 10$), indicating greater production and/or deposition or organic material nearer the entrance to the broad. Of the mineral fractions, the silt content was slightly greater with increased distance away from the inlet, in a similar manner to the organic matter. There was also a slightly greater proportion of the sand fractions closer to the inlet compared to further into the broad. This pattern of relatively greater particle sizes near the inlet suggests that a greater water velocity in this area may be acting to selectively transport smaller particles away in the water currents, thus causing a slightly greater mean particle size.

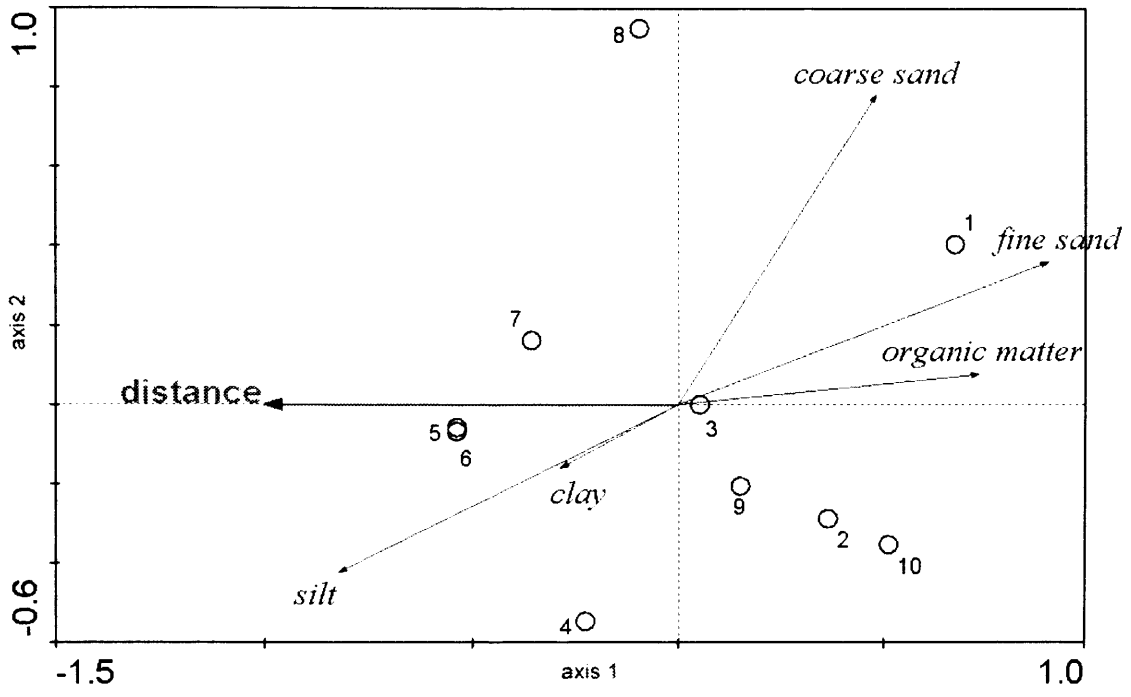

Figure 5.13 RDA triplot of Ranworth Broad sediment character with distance from inlet

Redundancy analysis of the sediment character data from each sample point found that 69.5 % of the total variance in this data was explained by distance from the inlet. Furthermore, a Monte Carlo permutation test showed that the influence of distance was highly significant $p = 0.005$. In Figure 5.13 the arrow describing the influence of silt upon the position of the sample points in the ordination space closest to distance from the inlet is that of silt content. As shown from the correlation analysis, this characteristic was found to be most closely related to distance, and also the arrows representing the sand fractions are pointing in the opposite direction to distance, supporting the observation that greater mean particle size occurred nearer the inlet.

Overall the low variability in the detected organic biocide concentrations and lack of a clear distributional pattern, in both the sediment and water compartments, indicates a relatively even spatial distribution of organic biocides within this broad. Clearly the concentration of biocides associated with sediments were not significantly influenced by the variation in mean particle size across the broad. The absence of diuron and isoproturon in the water samples fits the pattern of the wider spatial survey sites sampled in August 2004, so is not an atypical observation.

Mixing of the water column brought about by the tidal rise and fall may assist the diffusive distribution of the organic biocides entering from the inlet, and thus produce even mixing throughout the entire broad (Phillips *et al.* 1991). Wind induced waves are also capable of thorough vertical mixing of the water column in such shallow lakes (Sondergaard, Kristensen, and Jeppesen 1992;Cozar *et al.* 2005;Herb and Stefan 2005), especially in lakes that have little or no submerged macrophytes, such as Ranworth Broad (Pitt *et al.* 1997;Broads Authority 2004).

Contaminants in aquatic ecosystems generally have greater mobility in the aqueous phase compared to particulate bound contaminants (Knezovich, Harrison, and Wilhelm 1987). As the inlet represents the major hydrological connection to the river system, organic biocide contamination is predicted to enter via this route. A gradient in biocide concentration declining with distance from the inlet was however not observed in the dissolved or sediment bound Irgarol or atrazine. The lack of any clear triazine distribution in the sediments suggests mixing of the deposited sediments has also occurred. Alternatively the sediment concentrations may be the result of widespread in-situ partitioning to sediments from biocides contained within the well distributed dissolved phase. A combination of both mechanisms is probably

occurring, although the data presented here does not allow a distinction to be made between either of the processes.

The field-based solid-water distribution coefficient (K'_d) values of Irgarol and atrazine from the Ranworth Broad samples (57 and 12 respectively, see Table 5.2) were relatively low compared to the mean values from all the other spatial survey sites sampled in August 2004.

Table 5.2 Field based K'_d values from Ranworth Broad sample points.

		Irgarol	Atrazine
K'_d (l kg^{-1})	Mean	56.8	11.7
	S.D.	18.5	1.6
	n	9	9

This was especially so for Irgarol, which had a mean K'_d of 127 from the wider survey (Table 4.2). Ranking of the K'_d values from each River Bure sample site places Ranworth as the second and third lowest for Irgarol (n = 21) and atrazine (n = 27) respectively. The mean %LOI value for the Ranworth samples was 17.3 ± 3.6, which occupies the median of %LOI values from all 32 August 2004 sample sites. The lower mean K'_d value cannot therefore be attributed to a lower potential adsorption capacity through unusually organic matter content. Relatively low K'_d values indicate that the triazines were predominantly present in the water compartment in Ranworth Broad, which is supportive of these compounds being mainly transported in solution. This observation partly explains the presence of aqueous phase Irgarol within connected broads where direct exposure to AFP biocides released from passing vessels does not occur.

In line with the results from the wider August 2004 survey results, there were no significant relationships between organic biocide partitioning (K'_d) and any of the sediment characteristics measured. This pattern indicates a relatively even triazine distribution between water and sediment across the broad. However, the relatively low number of sample points and the small range of values observed within the variables has inevitably reduced the statistical power of such tests.

This transect study shows that within a non-navigable broad, connected to the river system, organic biocides are widely and relatively evenly distributed within both

aqueous and surface sediment compartments. A combination of tidal water movements and wind-induced waves are thought to assist the diffusive mixing of such biocides which has the net result of transporting such contaminants away from their originating sources, as observed at Ranworth Broad.

In addition to the organic AFP biocides, organotins were also analysed from sediments collected at each of the 10 sample sites. TBT concentrations were quantifiable in all samples in the range 71 - 225 ng g^{-1}. DBT was quantified from six of the sample points in the range 37 - 81 ng g^{-1}. The sample points with no detectable DBT were numbers 5 – 8, at the south-east end of the broad (Figure 5.14).

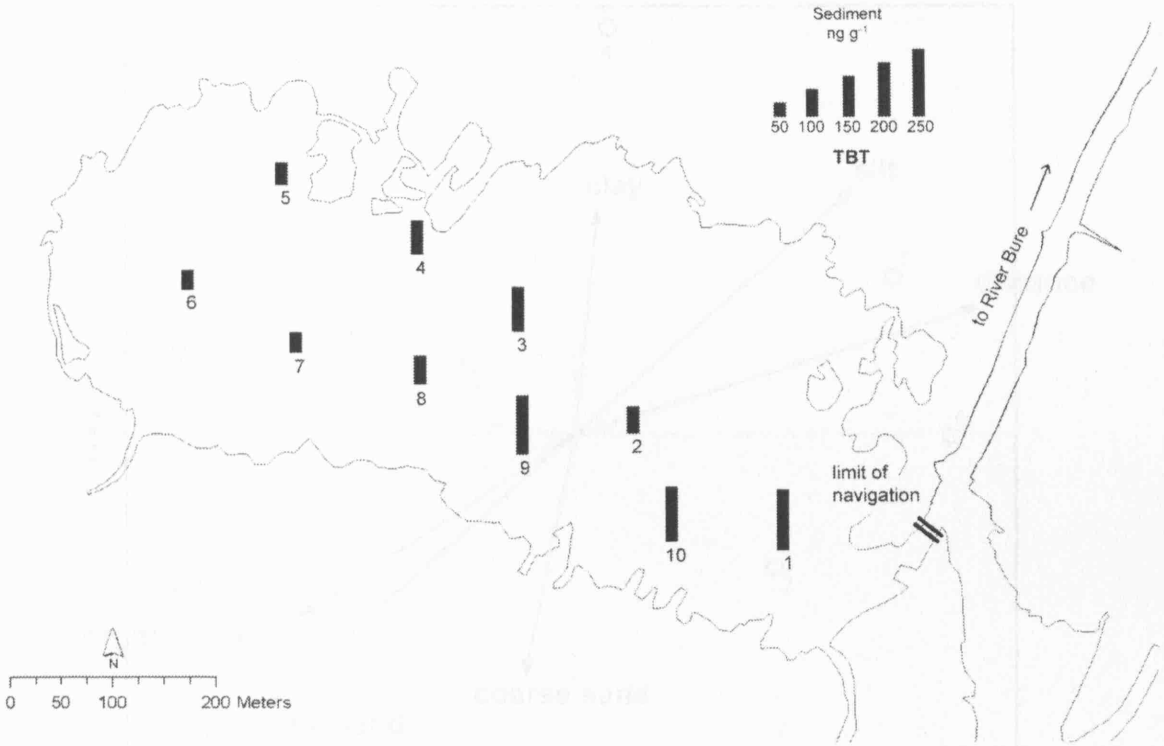

Figure 5.14 TBT sediment concentration within Ranworth Broad.

Within the Ranworth Broad organotin data, TBT and DBT had a significant positive relationship to each other (r^2 = 0.923, p = <0.001), as with the results from the wider catchment study (Figure 5.5). Sediment concentrations were significantly greater nearer to the inlet for both TBT (r = -0.796, p = 0.005) and DBT (r = -0.823, p = 0.003) as can be seen by the higher TBT concentrations in Figure 5.14 close to the inlet.

The ordination triplot in Figure 5.15 shows that the closest relationship between TBT and the environmental variables was with organic matter and fine sand content. Of the two, the relationship between TBT concentration and the fine sand fraction is greater, as indicated by the longer arrow, and also by a significant positive correlation ($r = 0.740$, $p = 0.014$). These two sediment characteristics were those most closely correlated with distance from the inlet, being present in greater amounts near to the inlet. However, the relationship between fine sand and TBT concentration may have been one of co-variance, as quartz sand has relatively few active sites upon which TBT can bind (Hoch and Schwesig 2004). The relationship between TBT and distance from inlet is clearly strongly negative as their respective arrows are at nearly 180° (pearson correlation; $r = -0.796$, $p = 0.006$).

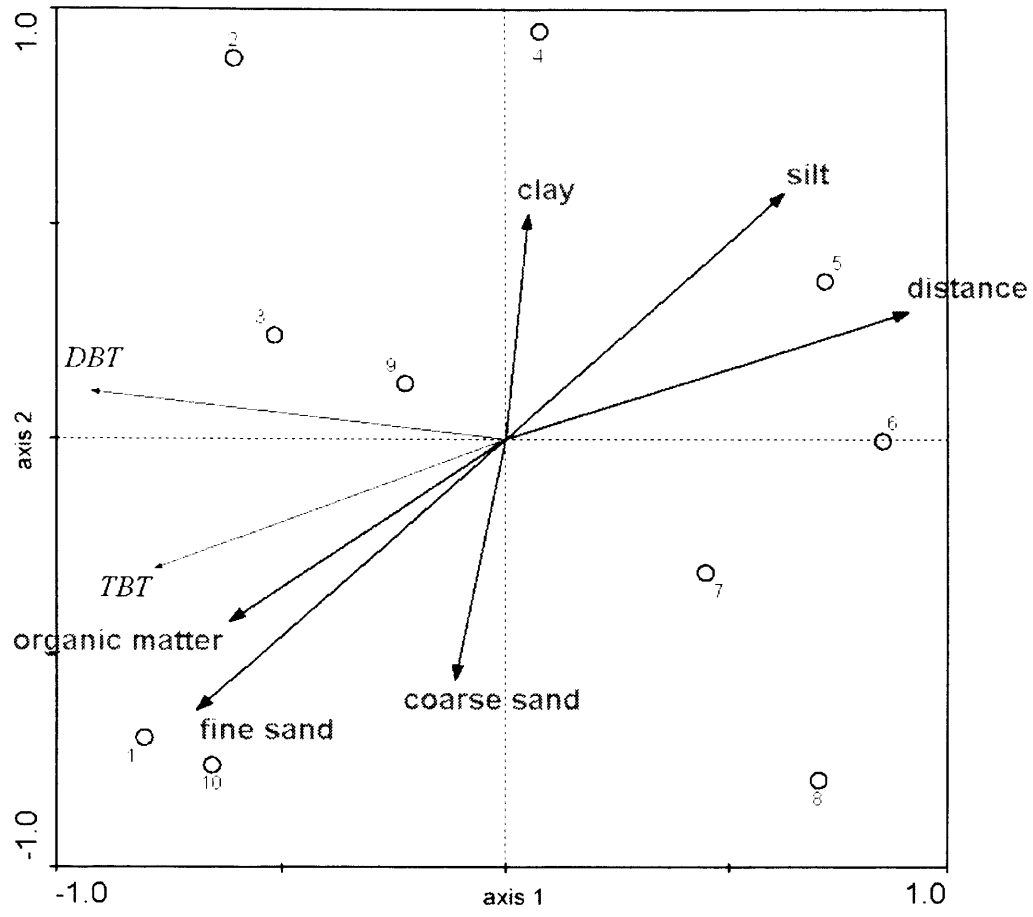

Figure 5.15 RDA triplot of Ranworth Broad organotin concentrations with sediment character and distance from inlet

Previous work has shown a positive distributional relationship between TBT in sediments and organic matter content (Hoch and Schwesig 2004;Buggy and Tobin

2006). However, in Ranworth Broad this relationship was close, but not significant, suggesting that several parameters influenced TBT distribution which were not fully quantified using the sampling approach adopted. The low variance explained by axis 1 of 14.9% also suggests that there was insufficient evidence to draw firm conclusions as to the processes explaining TBT distribution within Ranworth Broad.

The observed gradient in TBT sediment concentrations, from east to west across Ranworth Broad, is in contrast to the relatively even spatial distribution of the organic biocides. Given the greater preference of TBT to bind with sediment compared to organic biocides, the decrease in TBT concentration with distance from the inlet possibly reflects a gradient caused by TBT contaminated sediment being transported into the broad via the inlet. More intense sampling would possibly reveal the dominant sorption and transport mechanisms operating at Ranworth Broad, but an outline of the pattern of contamination has been revealed.

5.4 Organotin stratigraphy in broads sediments

In the present study four short sediment cores from the Broads were analysed for organotin contamination. The main focus of the present study was on sediment cores from Salhouse Broad (SALG1) and Hoveton Great Broad (HGBO1) on the River Bure, which were analysed for a range of biological proxies using palaeolimnological methods, see Table 2.3. This palaeoecological work follows and expands upon initial research by (Sayer *et al.* 2006) who analysed a sediment core for organotins from Wroxham Broad (WROX2), a navigable site downstream of the popular tourist centre of Wroxham village. The author also determined organotin profiles from cores collected in Hickling Broad (HICK1) , on the River Thurne (results included in Sayer et al 2006), and Barton Broad (BART9), on the River Ant. See Figure 1.2 for the study area map and Figure 2.10 for core locations within each broad.

5.4.1 TBT contamination history from selected River Bure broads

Core SALG1 sediment samples analysed for organotins (n = 17) had quantifiable TBT concentrations from the uppermost sample analysed, at 1 cm depth, down to 43 cm, with concentrations in the range 14 - 748 ng g^{-1} (Figure 5.16). In Figure 5.16, the extent of the grey bar from the origin represents the TBT concentration and the extent of the black bar from the origin represents DBT concentration. DBT was quantifiable from 1 cm down to 37 cm in the range 9 - 200 ng g^{-1}. MBT was not analysed from core SALG1. Samples analysed from 50 and 65 cm depth had no quantifiable butyltin species (see x notations to the left of the y axis in Figure 5.16 representing depths with no detectable butyltin). This compares well with results from core WROX2 that had quantifiable TBT concentrations between the depths 1 cm - 27 cm, in the range 117 - 791 ng g^{-1} (Sayer *et al.* 2006). Butyltin results from Hoveton Great Broad (n = 18), a broad connected to the River Bure, but not open to navigation, had much lower contamination than both Wroxham and Salhouse Broads. TBT concentrations were first quantifiable at 9 cm depth and extended down to 24 cm, in the range 50 - 178 ng g^{-1}. DBT concentrations were only quantifiable from the 18 and 21 cm depth samples at 30 and 48 ng g^{-1} respectively. MBT was analysed for core HGBO1 but was not detected in any sample. No butyltin species were quantifiable in samples from the uppermost 9 cm or below 25 cm depth.

The results of the radiometric dating of the cores SALG1 and HGBO1 are given in Appendices 9.3.1 – 9.3.2. Results of the radiometric dating of core WROX2 are given in Sayer et al (2006). The cores collected from the Bure broads were characterised by an extremely low activity of unsupported ^{210}Pb, which meant dating using conventional ^{210}Pb methods was not possible. The dilution of the normal atmpospheric flux of ^{210}Pb was predicted to arise from the relatively high sedimentation rates observed within each of these cores (see Tables 9.10 and 9.12). However, a distinct peak in ^{137}Cs activity, related to the 1963 peak in nuclear weapons testing, was observed within each core, and has been used as a reference date.

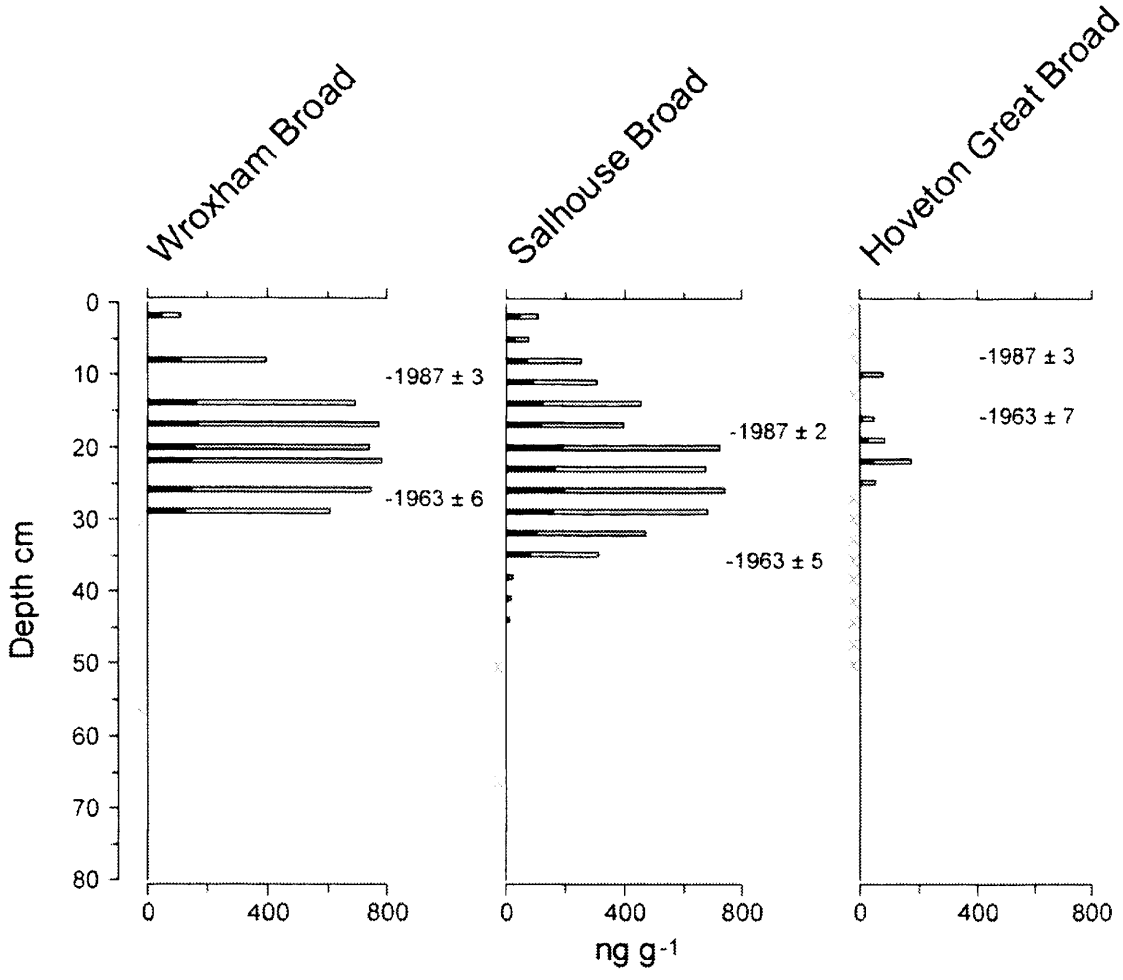

Figure 5.16 TBT (grey bar) and DBT (black bar) concentration profiles from Bure broads sediment cores.
x denotes no TBT detected, bars lengths are not additive
(Wroxham data from Sayer *et al* 2006).

The date of initial TBT contamination within WROX2 and SALG1 appears to be at a similar depth to the respective ^{137}Cs peaks, dated as approximately 1963. This date

of initial contamination is in accord with the stratigraphic pattern of TBT contamination observed in Lake Lucerne, Switzerland reported by (Fent *et al.* 1991). Within core WROX2 TBT first appears very suddenly, whereas within SALG1 there is a slight mixing of low TBT concentrations down core from the first relatively highly contaminated sample at 34 cm depth. The problems associated with sediment mixing in shallow lakes however has not prevented use of sediment records from these environments for use in monitoring and environmental reconstruction (Anderson and Odgaard 1994). Fent *et al* (1991) also show a mixing down of TBT below the 1963 ^{137}Cs peak, beneath a TBT concentration peak that was roughly a quarter of the maximum TBT concentration observed in SALG1 and WROX2. TBT concentrations were also observed below the 1963 ^{137}Cs peak in core HGBO1. However, the radiometric dating of HGBO1 was the least reliable of the cores from the Bure broads, as the ^{137}Cs activity was very low, with a peak of only 7.8 Bq kg^{-1} compared to 19.3 and 16.0 Bq kg^{-1} from SALG1 and WROX2 respectively. An increased resolution of sample depths analysed would have given greater confidence in these calculated dates. However, given the generally low activity levels, use of a complementary sediment dating technique, such as the enumeration of spheroidal carbonaceous particles (SCPs), may have given more robust dating (Rose and Rippey 2002).

The similar pattern of butyltin concentrations declining after the predicted 1987 date in cores SALG1 and WROX2, representing the decrease in TBT input following the ban on TBT usage on boats <25 m, supports the radiometric chronologies determined for these two cores. The ^{137}Cs profiles were well resolved and of sufficient activity levels to have a greater confidence in the predicted dates, compared to core HGBO1. Persistence of butyltin concentrations in the surface sediments of WROX2 and SALG1 most probably reflects the continual disturbance and mixing of contaminated surficial sediments in these shallow lakes and possibly continued release of TBT from boat hulls following the 1987 ban. There is likely to have been a period of time after the ban when boats still coated with TBT containing AFPs were present in the Bure waterways. This could have arisen either through active reapplication of TBT containg AFPs, or simply through no other coating being applied on top of the old AFP to prevent the continued leaching of TBT.

These butyltin profiles (Figure 5.16) clearly demonstrate the lower level of TBT exposure experienced within the non-navigable Hoveton Great Broad. Despite the

relatively less intense contamination observed within HGBO1, the known period of active TBT usage in AFPs, between the mid-1960s and 1987, is defined more clearly as a distinct contamination event in this core. In comparison, the TBT profiles of SALG1 and WROX2 display prolonged contamination after the 1987 date. In-situ mixing, continued leaching from boat hulls and incorporation of contaminated sediment transported from elsewhere in the River Bure waterway has clearly extended the period that TBT was detectable in SALG1 and WROX2. The profile in core HGBO1 suggests that only during the peak period of its usage was sufficient TBT incorporated in deposited sediments, at the core site, to persist in quantifiable concentrations to the present day. There also appears to have been minimal deposition of TBT contaminated sediment transported into the broad following the 1987 ban. The lack of detectable TBT concentrations or any of the breakdown products, in the top 9 cm, or within either of the surface sediment samples collected during April 2003 and August 2004 supports this inference.

5.4.2 TBT contamination history of Hickling and Barton Broads

TBT concentrations (n = 13) in core HICK1 (Hickling Broad) extended from the uppermost sample at 1 cm, continuously down to 12 cm, within the range 55 - 102 ng g^{-1} (Figure 5.17). DBT was quantifiable from the same depth levels in the range 19 - 32 ng g^{-1}. MBT was also analysed for, but none was detected from the HICK1 samples. Radiometric dating of HICK1 was relatively successful, with significant activity levels of ^{210}Pb extending down to 20 cm depth. There was also a well resolved ^{137}Cs profile, peaking at 15 cm depth, representing a predicted date of 1963. This was further supported by detection of traces of ^{241}Am, a rarer artificial fallout radionuclide whose atmospheric concentration also peaked in 1963. One sample at the 27 cm depth level had a trace amount of TBT quantifiable at 13 ng g^{-1}. The likelihood that this contamination occurred at the time of sediment deposition is slight, as the sample depth pre-dates 1922, which is before TBT was first synthesised (Bokranz and Plum 1975). A small amount of cross contamination with more recent sediment either during sampling, extraction or analysis is therefore suggested as the most likely cause of this erroneous result.

The level of butyltin contamination within HICK1 was clearly lower than within the navigable Bure broads, presumably as a result of historically lower boat numbers/movements within Hickling Broad (Figure 2.1). Additionaly, the core

location was over 500 m away from the navigation channel where most of the boat activity is concentrated (Figure 2.10). The relatively low depth that butyltins extend down core in HICK1 is due to the relatively lower sedimentation rate observed within this core (Table 9.14). The initial date of TBT detectable in the sediment of core HICK1 is slightly later that observed in WROX2 and SALG1, being predicted to have been at around 1969. Butyltin concentrations do not appear to have decreased significantly above the 1987 sediment depth, as they did in the Bure broads cores.

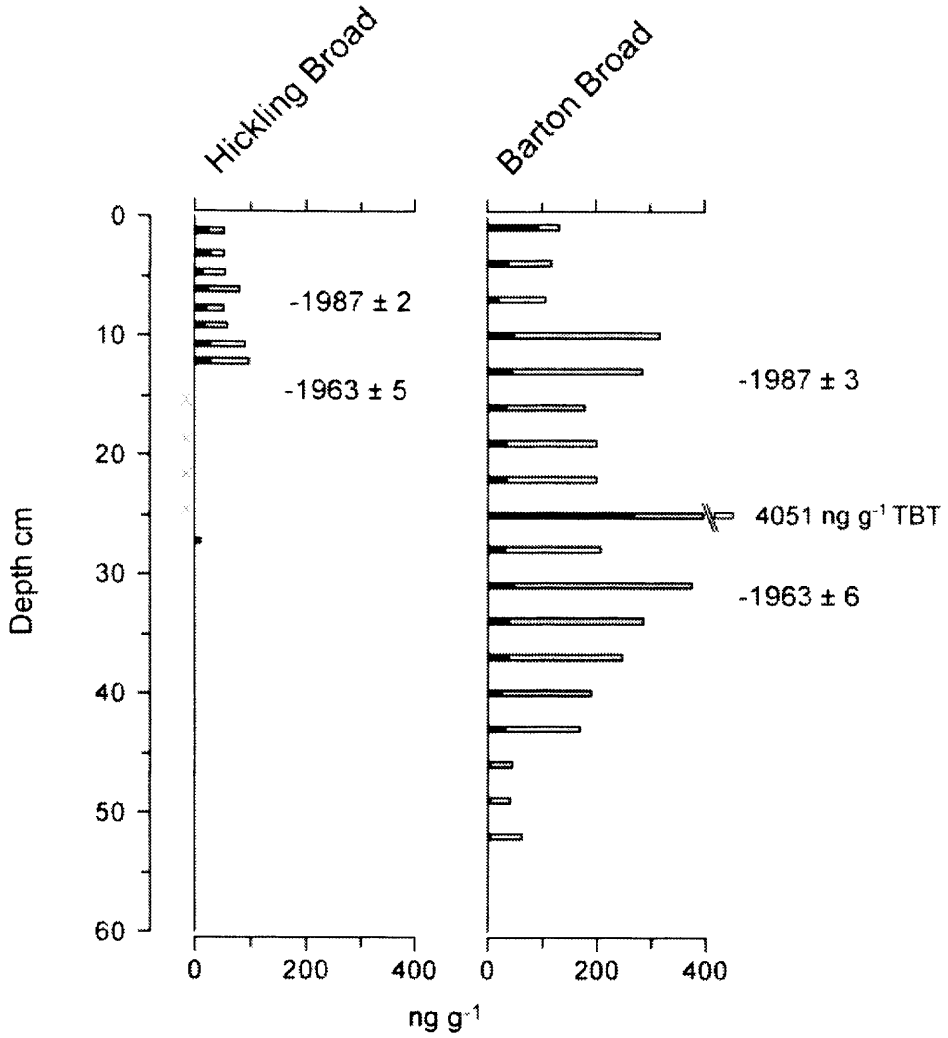

Figure 5.17 TBT (grey) and DBT (black) concentration profiles from dated Hickling (HICK1) and Barton (BART9) sediment cores
x denotes no TBT detected, bars lengths are not additive

Core BART9 collected from Barton Broad had TBT concentrations (n = 18) that extended from the uppermost sample level at 0 cm down to 51 cm within the range 46 - 4051 ng g^{-1} (Figure 5.17). DBT and MBT concentrations both extended down to

42 cm within the range 30 - 271 ng g^{-1} and 14 - 68 ng g^{-1} respectively. ^{210}Pb dating of core BART9 proved difficult, due to low unsupported ^{210}Pb activity. A ^{137}Cs peak was observed at 30 cm depth (Figure 9.7c), but the maximal value was only slightly higher than the activity in the remainder of core, which was relatively constant to the surface. Detectable concentrations of all three butyltin species extended down to a depth of 43 cm, which is well below the 1963 ^{137}Cs peak. Even given the dating error associated with this predicted date, which is relatively wide, incorporation of butyltins has clearly occurred earlier in the sediment sequence than in any of the other cores analysed. The location of core collection may have played an important role in the observed patterns of the BART9 ^{137}Cs and butyltin profiles. BART9 was collected from only 10 m out from the broad margin, rather than in the preferred coring area of the central or deeper basins of lakes, where greatest sediment focussing occurs, and less sediment disturbance is experienced (Hilton, Lishman, and Allen 1986). Restoration measures, which included the dredging of surficial sediment from the entire broad, were in operation at the time of coring. This was part of the Barton Clearwater 2000 project where suction dredging was carried out from 1996 to 2001 (Broads Authority 2006). This meant that only a small area of undredged sedimentary material remained where collection of a core was possible, thus limiting suitable locations.

Wave action and/or bioturbation in the shallower marginal area may have caused excessive mixing of the sediments during deposition, which would produce a smoothed profile of contaminants such as ^{137}CS and the butyltins. The high level of butyltin contamination quantified from the BART9 sample at 24 cm depth, was anomalous for all the core material analysed as part of the present study. No replicate analysis of this sample was conducted, so the significance and cause of the high concentrations, such as the possible presence of a TBT containing AFP paint flake within the analysed sediment, can only be surmised. Given the doubts over the reliability of the radiometric dating from this core and the observation of butyltins much deeper in the buried sediments than expected, the results from core BART9 cannot be used as a good indicator of temporal variation in TBT exposure. It does however show that significant TBT contamination occurred in Barton Broad and highlights that core collection location is important in studies of contaminant history.

5.4.3 TBT degradation with depth

Where TBT and DBT were quantified from the same depth level within the sediment cores, the degradation ratio of TBT/DBT was calculated (Table 5.4). Figure 5.18a shows that WROX2 and SALG1 had similar TBT/DBT ratio profiles down core, suggesting that the mechanisms influencing degradation of TBT were similar.

Table 5.3 Summary of TBT/DBT values for broads sediment cores.

	min	max	mean	± 1 S.D.	n
WROX2	2.2	5.1	4.2	1.0	8
SALG1	2.2	4.5	3.3	0.6	13
HGBO1	2.9	3.7	3.3	0.5	2
HICK1	1.8	3.2	2.7	0.5	8
BART9	1.4	15.0	5.9	2.9	15

Both cores show a reduction in this ratio, from approximately 20 cm upwards. (Dowson *et al.* 1993c) reported that measureable TBT degradation was only observed in the aerobic sediment zone during a long-term laboratory study on spiked freshwater sediments.

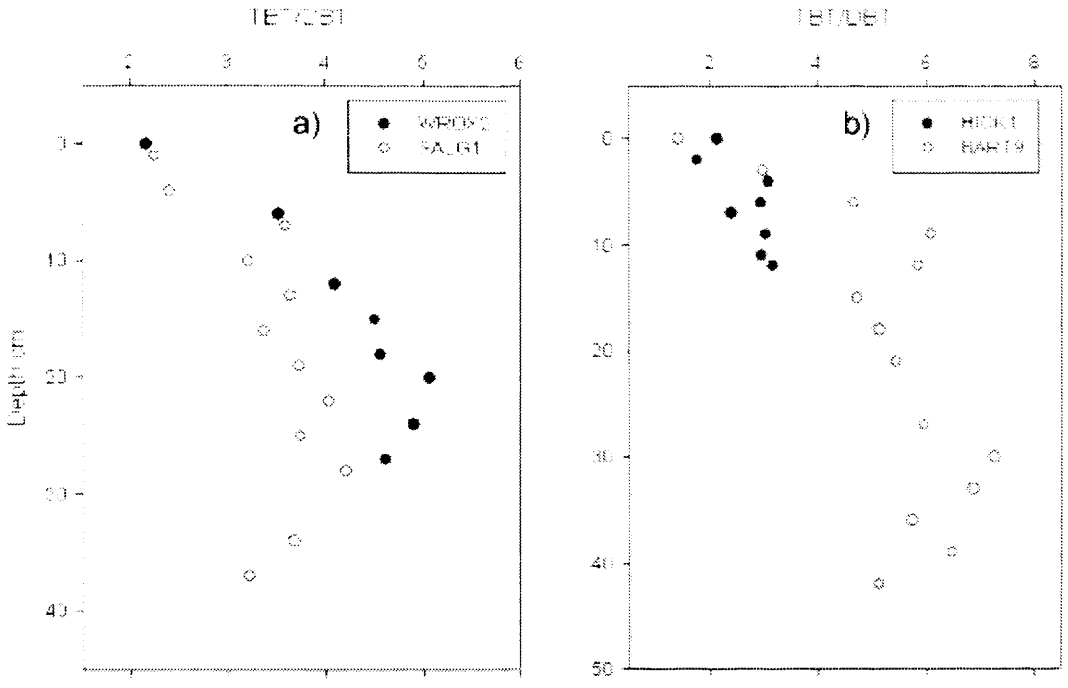

Figure 5.18 TBT/DBT ratio with depth in four Broads sediment cores.
(TBT/DBT value of 15 at 24 cm excluded from BART9 profile, see text).

Studies in the Broads have shown that the aerobic zone in sediment extends down to about 3 cm, below which redox potential becomes highly negative (<100 mV) (Pitt

et al. 1997). In all the sediment cores analysed for butyltins, the greatest degradation of TBT was observed in these uppermost sediment layers, suggesting biodegradation of TBT by aerobic micro-organisms as the principal breakdown mechanism, as postulated by (Dowson *et al.* 1996).

Similar increases in the relative amount of DBT to TBT near the sediment surface were observed within HICK1 and BART9 (Figure 5.18b). The results from HICK1 however, show much lower variability in TBT/DBT with depth, whereas above 10 cm depth BART9 TBT/DBT declines rapidly. WROX2, SALG1 and BART9 all show relative stability in TBT/DBT below 10 cm, with all values > 3 suggesting minimal degradation below this depth. The peak period in TBT/DBT values in all three of these cores also corresponded to the respective peak period in TBT concentration. The high TBT concentrations present during deposition of this sediment may therefore have contributed to the increased persistence observed, through elimination of the micro-organisms which could have acted as biodegradative agents. This may be a similar mechanism which contributed to the relatively high TBT/DBT values observed within the boatyard samples (Figure 5.8), which coincidentally had the greatest TBT concentrations within the spatial survey (Figure 5.4).

5.5 Conclusions

TBT and its degradation products were found to be widespread contaminants in contemporary surface sediments of the River Bure waterway. TBT concentrations were significantly greater in boatyard sites compared to all other site types, although variation in the results within and between boatyard sites was relatively high. Maximum concentrations quantified in the present study are among the greatest reported from studies of TBT contamination in freshwater sediments from around the world. The relatively lower level of contamination in navigable sites and connected broads was dependent upon factors such as degree of hydrological connectivity and the relative amount of boating activity at each site. Conversely, no butyltins were detected at any of the river or broad sites isolated from navigational activity, or in sediments downstream of WWTW discharges, which have been shown to represent a potential alternative source of butyltins to the aquatic environment. These findings are in accord with previous work which identified AFP leachate as the primary source of TBT in this area (Waite *et al.* 1989;Dowson *et al.* 1992). A distinct environmental

contamination gradient of TBT therefore remains within the surface sediments of the River Bure waterway, with mean concentrations declining between site types in the order boatyard>navigable=connected>isolated. This relative contamination pattern between site types was exactly the same as observed within the Irgarol concentration data (Chapter 4, Figure 4.6). Mulitvariate analysis of the butyltin concentrations and site specific environmental variables showed that site type had the greatest influence over variability in TBT concentrations, over and above the characteristics of the surface sediment. The spatial pattern of TBT concentration observed in the study area was therefore predominantly determined by the amount and type of boating activity at each site.

The present study also demonstrated the high environmental persistence of TBT in the Broads sediments, as over 16 years had elapsed since TBT was banned in AFPs on boats < 25 m. Greatest persistence of TBT was observed in boatyards, as inferred from the ratio of TBT/DBT. Comparison with the limited historic data available showed that sediment in the River Bure boatyards was generally less contaminated than in the past, however the significance of this is unclear as very little previous data was available. Surprisingly, very little difference could be found between past and contemporary TBT concentrations outside of boatyards, i.e. between navigable and connected sites. This analysis was more robust than the comparison of boatyard data from different time periods, as a greater amount of previously reported data was available from the navigable and connected site types. Transport of TBT from highly contaminated "hotspots" is predicted to account for the relative lack of reduction in average concentration outside of the boatyards.

The transport of AFP biocides from source areas to non-navigable, but hydrologically connected broads was demonstrated by the spatial survey and the the Ranworth Broad transect study. TBT was detected in surface sediments from three of the five connected broads sampled, including Ranworth Broad. Within this broad, a declining gradient in TBT sediment concentration with distance from the entrance dyke was observed. For the more soluble triazine biocides studied, Irgarol and atrazine, their distribution was relatively homogenous throughout the broad, both within dissolved and sediment phases.

The temporal variability of TBT contamination experienced within the Bure broads was determined through analysis of sediment cores. Relatively undegraded TBT

was quantified within the buried sediment, which produced clear pollution profiles that also demonstrated the historic differences in relative exposure to TBT between site types. The connected Hoveton Great Broad was shown to have been contaminated during the peak period of TBT usage. However, evidence of this pollution was not present in the uppermost sediment layers of the core analysed, whereas in the cores collected from Wroxham and Salhouse Broads, vertical mixing and transportation of contaminated sediments has meant that TBT was still detectable up into the surface sediments. The core location was shown to be an important factor in obtaining a clear sediment stratigraphy from shallow lakes.

CHAPTER 6 – PALAEOLIMNOLOGY OF TBT EXPOSED BROADS

This chapter outlines the results obtained from the palaeolimnological analyses performed on sediment cores collected from Salhouse and Hoveton Great Broad on the River Bure, and Hickling Broad on the River Thurne. Analyses included the identification and enumeration of macrofossil and cladoceran remains; characterisation of sediment lithology; and quantification of total phosphorus, total nitrogen and sediment organic matter δ^{13} carbon profiles. An assessment of reported toxicological test data is also presented with the aim of determining whether soluble TBT concentrations present in the Broads during its active usage would have been sufficient to cause the ecological changes observed.

6.1 Salhouse and Hoveton Great broads – Bure broads

Total length of sedimentary material in the wide diameter core tubes, prior to extrusion, were 88 cm and 87 cm for SALG1 and HGBO1 respectively. Upon extrusion a slight compaction of the sediment was observed, as the lowest recorded depth sampled was 2 cm short of the original length from both cores. A total length of 185 cm was achieved for core HGBO2 using the Livingstone corer. Minimal compaction during extrusion was observed with only 1 cm being lost over the extrusion of 164 cm. Extrusion was stopped at this depth in HGBO2 as solid peat had been reached.

The similar core lengths achieved using the wide diameter corer were considered to be its maximum operable depth in such sediments. In smaller diameter corers, the vacuum above the sediment surface and friction between sediment and the tube walls help maintain the sediment within the corer. However, with the wide diameter corer used in the present study, a far greater sediment weight was present beneath the core head vacuum, and less friction with the tube walls was present per volume of sediment sampled (smaller tube wall surface to sediment volume ratio). Fortunately, the lower sediments reached were relatively compacted and this acted to plug the bottom sediments into the corer and prevented major loss of material from the core bottom.

In Hoveton Great Broad the upper peat is characterised by a high organic matter content (~80%) with virtually no carbonate present within the peat (Figure 6.1). The

peat included small pieces of solid wood and numerous fibrous remains and was almost black in colour. At 146 cm the solid woody material had disappeared but the fine peat fibres remained. This was the start of a transition stage between basal peat and the beginning of the lake sediments. In a previous study which collected multiple cores, the top of the peat layer in Hoveton Great Broad was found to range between a sediment depth of 100 –140 cm (Moss 1988). At 142 cm depth in HGBO2 freshwater mollusc remains appeared for the first time and the sediment had become browner and fewer peat fibres were present. The sediment composition profiles show marked changes during this stage with a rapid increase in carbonate and decrease in organic matter content.

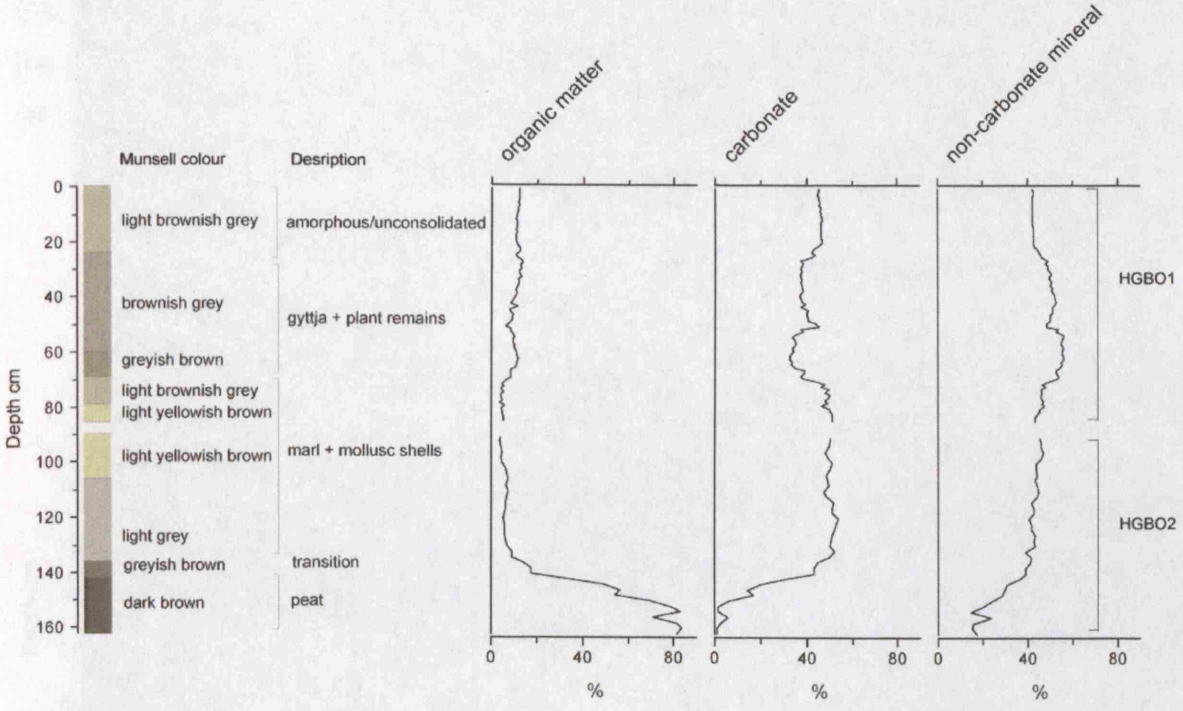

Figure 6.1 Hoveton Great Broad core descriptions and sediment composition (joint results from cores HGBO1 & HGBO2).

The lake sediments then entered a relatively stable period between 136 – 70 cm, with carbonate content around 50% and < 10% organic matter. This gritty textured marl material was also characterised by abundant mollusc shells. Both HGB01 and SALG1 (Figure 6.2) collected with the wide diameter corer extended down into this layer, which was rich in the carbonate deposits that characteristically encrust charophyte stems. On the encrustations the spiral structure of the charophyte internodal cell cortex were still visible. This distinctive layer was presumed, from

comparison with previous reports of cores collected from Hoveton Great Broad, to be representative of a period of macrophyte dominance (predominantly charophytes) (Stansfield *et al.* 1989).

At around 70 cm depth in both HGBO1 and SALG1 carbonate content decreased sharply, with concomitant increases in organic matter and non-carbonate mineral content. In both of these cores, the previously paler coloured sediments became darker with the abundance of visible mollusc shells reduced to sparse fragments. The depth that carbonate content and abundant mollusc shell remains decreased, matched that observed by Moss (1988) in Hoveton Great Broad. In HGBO1 the organic matter content remained at this higher level through to the top of the core. In SALG1 organic matter decreased to relatively steady levels, but with values below the peak observed at 70 cm. These darker sediments above 70 cm had a smooth, non gritty consistency.

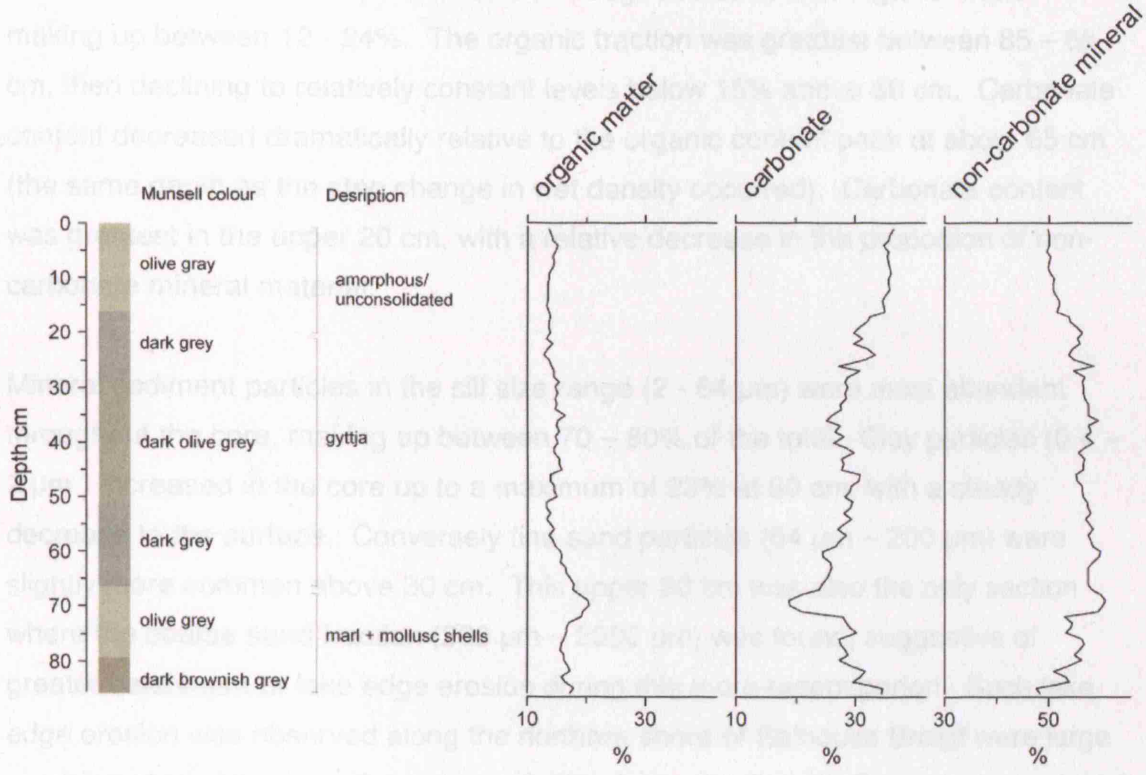

Figure 6.2 Salhouse Broad (SALG1) core description and sediment composition.

Toward the top of each core, the carbonate content profiles began to increase, in HGBO1 and SALG1 from 25 cm and 30 cm respectively, matched by a decline in the non-carbonate mineral content. The upper 20 cm of both cores was made up of

amorphous, unconsolidated sediment with very little visible plant or other biological remains. The top 5 cm of both cores were very fluid.

6.1.1 SALG1 sediment stratigraphy

The most comprehensive range of analyses used to characterise sedimentary material was performed on core SALG1. Percentage sediment dry weight (Figure 6.3, first graph on the left), and its inverse metric, percent water content, show how the sediments in the top 10 cm were less consolidated and more fluid than the rest of the core. Water content in these very liquid sediments was in the range 80 - 85 %. Wet density was greatest below 65 cm but did not increase linearly with depth. This pattern suggests that changes in sediment composition may have influenced the wet density values to some extent and results were not solely influenced by sediment compaction at greater depth. Mineral material (carbonate + non-carbonate) dominated the sedimentary composition through the core, with organic matter making up between 12 - 24%. The organic fraction was greatest between 85 – 65 cm, then declining to relatively constant levels below 15% above 60 cm. Carbonate content decreased dramatically relative to the organic content peak at about 65 cm (the same depth as the step change in wet density occurred). Carbonate content was greatest in the upper 20 cm, with a relative decrease in the proportion of non-carbonate mineral material.

Mineral sediment particles in the silt size range (2 - 64 μm) were most abundant throughout the core, making up between 70 – 80% of the total. Clay particles (0.4 – 2 μm) increased in the core up to a maximum of 23% at 60 cm, with a steady decrease to the surface. Conversely fine sand particles (64 μm – 200 μm) were slightly more common above 30 cm. This upper 30 cm was also the only section where the coarse sand fraction (200 μm – 2000 μm) was found, suggestive of greater catchment or lake edge erosion during this more recent period. Such lake edge erosion was observed along the northern shore of Salhouse Broad were large numbers of tourists enter the water and disturb the sandy soil. Concentration of total carbon and nitrogen are both relatively high below 70 cm in SALG1, the values then both decline above this depth. The C/N ratio fluctuates around 10%, with slightly higher values about 50 cm. Organic carbon stable isotopic analysis (δ^{13} C) displays a trend of enrichment above 70 cm and a further step increase above 40 cm.

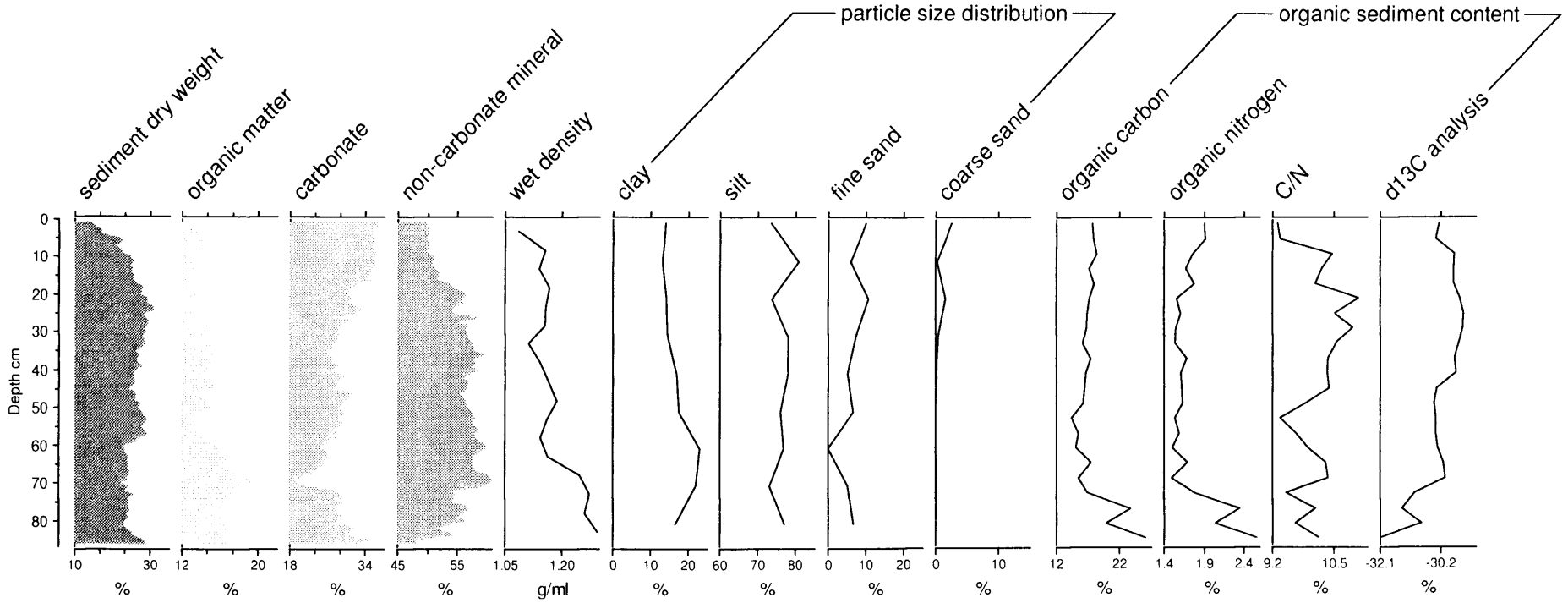

Figure 6.3 SALG1 sediment lithology.

6.1.2 Biostratigraphical results

Within this section the complete dataset for all macrofossil and cladocera remains identified from cores SALG1 and HGBO1 are given. The zonation in each of the following biostratigraphic diagrams is based on constrained clustering analysis for all the taxa represented in each individual diagram. Therefore the zone label numbers represent the major divisions in the classifications of each group of proxy remains and are not common between different proxy groups or cores. The radiometric dating results for cores SALG1, HGBO1 and HICK1 are given in Appendix 9.3 and described in section 5.4.1.

6.1.2.1 Salhouse Broad – Bure Broads

Of the macrophyte remains found in core SALG1 marginal and semi-terrestrial species, such as *Carex* sp. and *Juncus* sp. were eliminated from zonation analyses, but in-lake, swamp species such as *Schoenoplectus* sp. and *Typha* sp. were included (Figure 6.3). All of the submerged taxa were present in the lower section of zone I, prior to the decline in carbonate content at 70 cm depth. The most characteristic taxa present in zone I (86 – 52 cm) were *Chara* oospores, which rapidly declined above 70 cm. In the lower core (below 70 cm), there was a mixed flora, with species such as *Myriophyllum spicatum* (L.) and *Potamogeton pusillus* agg. were present alongside the charophytes. *Potamogeton pusillus* agg. incorporates the species *P. pusillus* (L.) and *P. berchtoldii* (Fieber). These species remained undifferentiated as separation is not possible from leaf tip material alone. Within zone II however, between 52 and 30 cm, very little diversity in submerged macrophyte taxa was present, with waterlily remains (Nymphaeaceae trichosclereids) dominant. Dating sggest that waterlilies started to become the dominant species some time after 1945. The peak in abundance of the waterlily leaf cells occurred in this middle zone. In zone II *Chara* oospores were also detected between 36 and 34 cm (in the early 1960s), though not at the concentrations found in zone I. Between 32 – 42 cm depth macrofossils were sampled at a greater intensity (every 2 cm), as it covered the part of the core where TBT was initially detected. In the uppermost section of the core above 30 cm, zone III, both waterlily and *Ceratophyllum* remains were present, but only sporadically. A low abundance of *Chara* oospores was found within zone III.

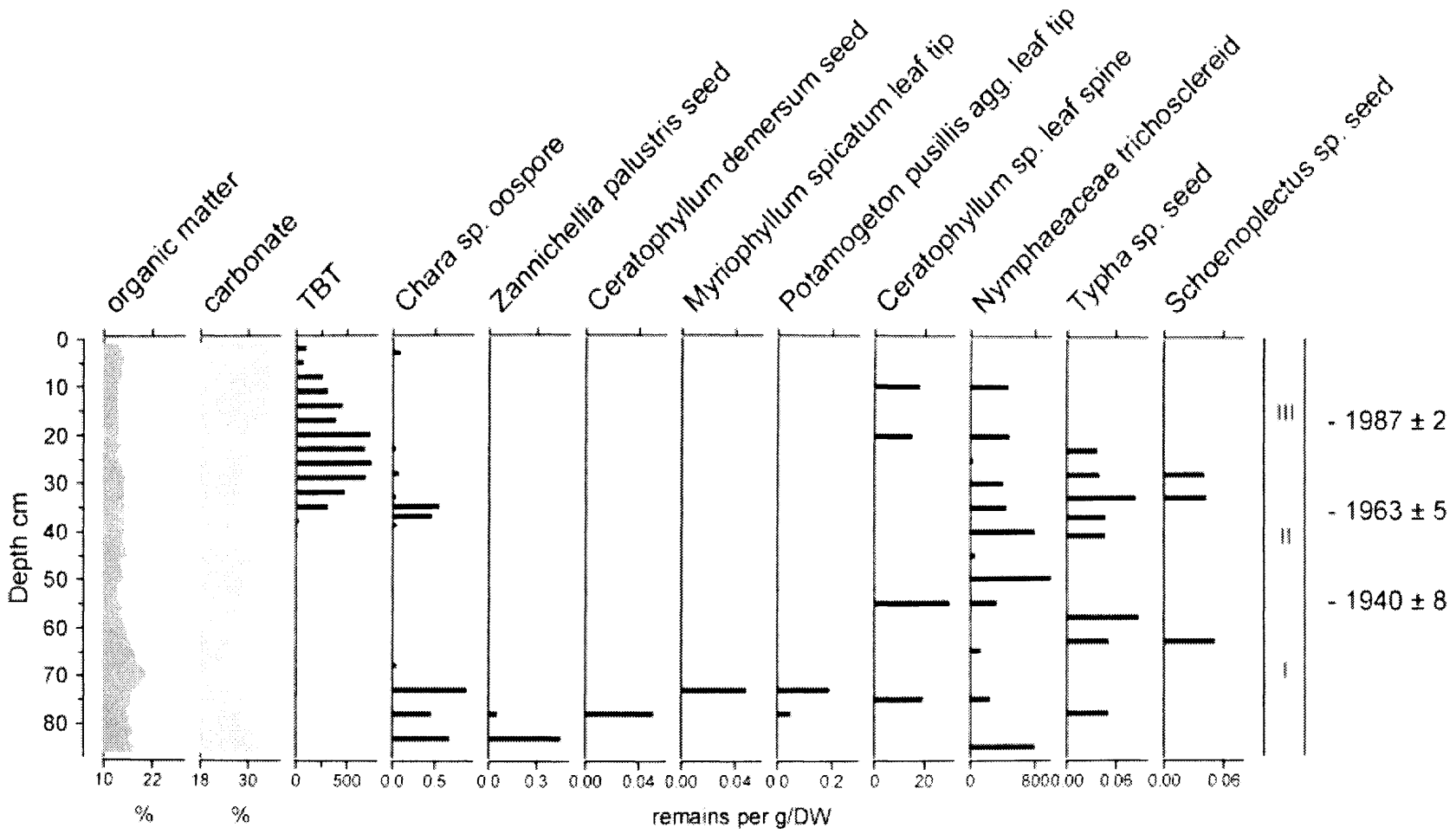

Figure 6.4 SALG1 plant macrofossil stratigraphy.

After the abundance of Chara oospores crashed (above 70 cm), many of the other submerged macrophyte species remains found alongside the *Chara* were not detected again in the core. Charophytes are particularly prodigious in oospore production when compared to the seed production of most submerged angiosperms (Zhao *et al.* 2006). Relative abundances of the remains of different macrofossil taxa therefore do not relate directly to the relative abundances of mature plants within the lake at the time of oospore/seed deposition. In a comparison of historical, macrofossil and pollen records of aquatic plants at Groby Pool, a shallow lake in the English Midlands (Davidson *et al.* 2005) found that macrofossils generally underestimated past species diversity, but provided a reliable indication of temporal change in the dominant taxa. The pattern observed in SALG1 therefore suggests that a relatively depauperate angiosperm flora replaced the stoneworts above 70 cm.

In core SALG1 a relatively high number of species were found (Figure 6.5). The lowest zonation, zone I, extends from the bottom of the core to 65 cm. Within this zone, the greatest abundance and diversity of mollusc taxa were present. The only species not present in zone I were *Potamopyrgus antipodarum* (Gray), a mud snail and *Dreisenna polymorpha* (Pallas), the zebra mussel. This is not surprising in that both were relatively recent introductions to the British fauna, having gained widespread distributions within the UK by 1930 and 1850 respectively (Kerney 1999). The depths of initial detection of these species is also supportive of the radiometric dating from this core. Above this bottom zone ending at 65 cm, total mollusc abundance and diversity crashed very suddenly. Between 65 - 45 cm (zone II), six taxa observed in zone I were not found again, including *Theodoxus fluvaitilis*, *Hippeutus complanata, Acroloxus lacustris* and *Planorbis carinatus/planorbis*. Those taxa that were present in zone II were *Bithynia tentaculata* (L.), *Valvata piscinalis* (Müller) and the immature stages of both *Valvata* and *Lymnaea* species. Zone III (45 – 30 cm) was characterised by a peak in abundance in the early 1960s of bivalve remains, notably *Anodonta/Anatina* shell fragments and *Dreisenna polymorpha*, as well as a small peak in *Potamopyrgus antipodarum*. Zone III ended at 30 cm, the same depth as the zone II/I transition in the plant macrofossil stratigraphy diagram, (Figure 6.4). Zone IV for the mollusc data (30 – 0 cm) showed an even greater reduction of species diversity, with 11 of the original 16 taxa not found in this top part of the core. The zone was coincident with the peak in TBT concentration. The most abundant taxa in zone IV were *Pisidium* sp, the pea mussels, with only a few individual remains of immature gastropod taxa.

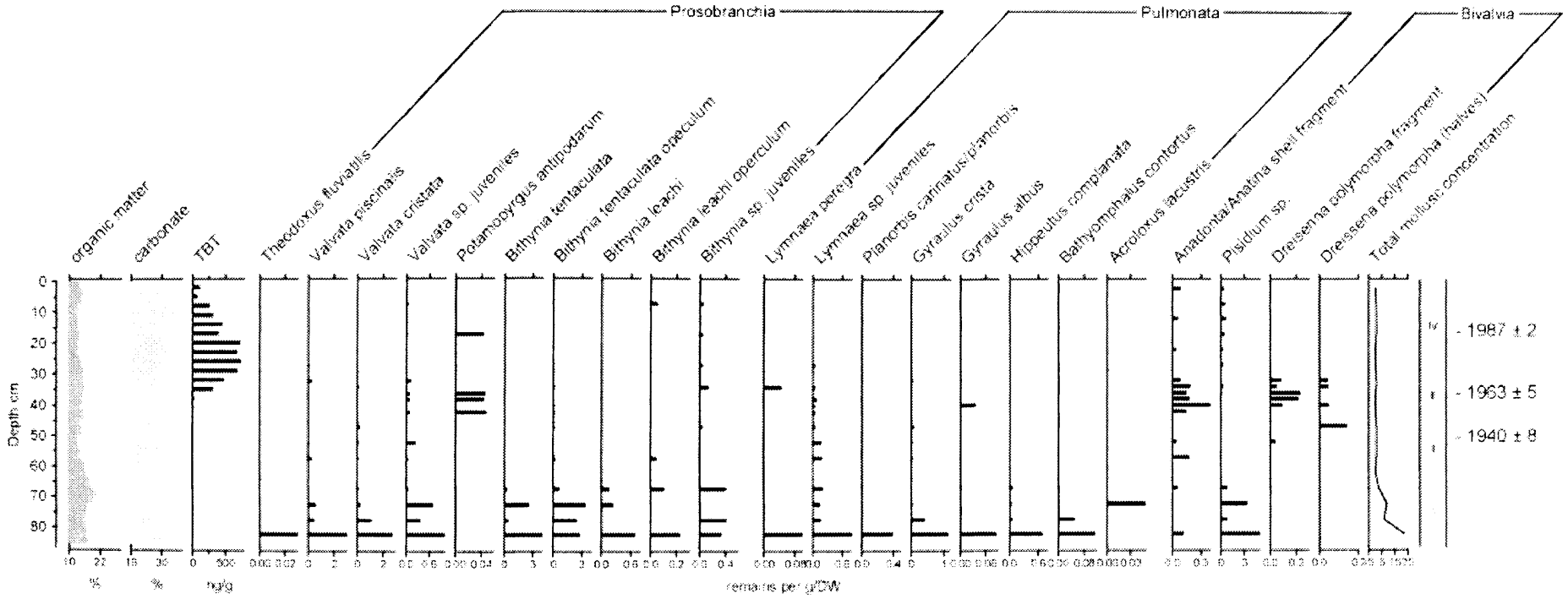

Figure 6.5 SALG1 mollusc stratigraphy.

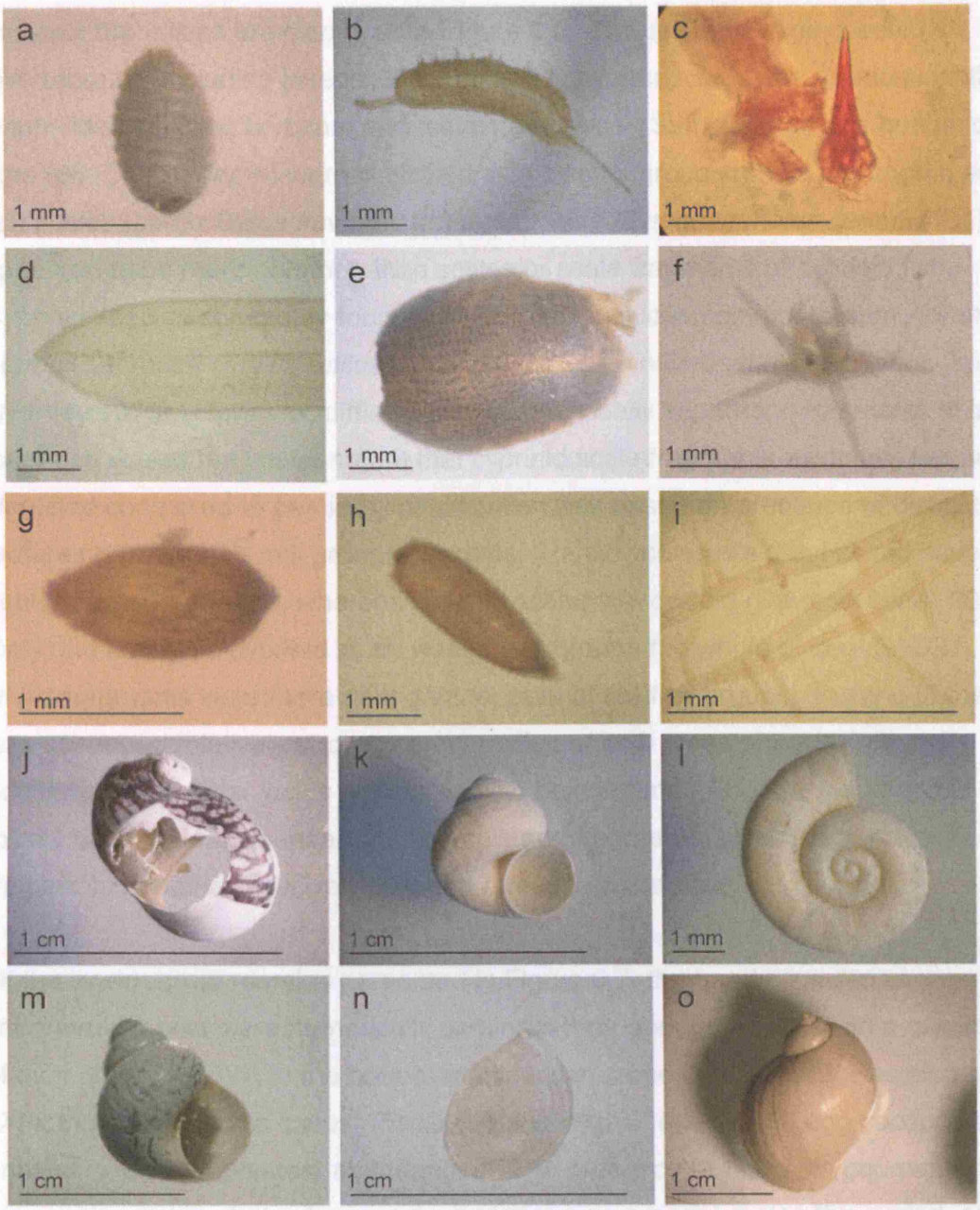

Figure 6.6 Examples of macrophyte and mollusc marofossil remains found in River Bure broads sediment cores.

(a) *Chara* oospore; (b) *Zannichellia palustris* fruit; (c) *Ceratophyllum* sp. leaf spine; (d) *Potamogeton pusillus* agg. leaf tip; (e) *Nymphaea* alba seed fragment; (f) Nymphaeaceae trichosclereid; (g) *Juncus* sp. seed; (h) *Typha* sp. seed; (i) Phragmites root; (j) Theodoxus fluviatilis shell; (k) Valvata piscinalis shell; (l) *Valvata cristata* shell; (m) *Bithynia tentaculata* shell; (n) Bithynia tentaculata operculum; (o) Lymnaea peregra shell.
(Photographs – D.J. Hoare)

The abundance of macrofossil remains from vertebrate and invertebrate (excluding mollusc) organisms are displayed in Figure 6.6. Groups represented were fish; invertebrates, including insects, leeches and flatworms; cladocera, predominantly Daphniidae species; bryozoa; and testate amoebae. Samples from the bottom of the core (86 - 70 cm depth) were classified as a distinct group (zone I). In zone I, percid fish scales (perch *Perca fluviatilis* (L.) and/or ruffe *Gymnocephalus cernuus* (L.)) appeared to be more common than scales or scale fragments of cyprinid fish. Cyprinid species commonly found in the Broads includes common bream *Abramis brama* (L.), roach *Rutilus rutilus* (L.) and rudd *Scardinius erythrophthalamus* (L.) (Wortley 1976). However, difficulties in taxonomically separating fragments of sub-fossil fish scales may have meant that cyprinid scale fragments were less frequently identified compared to percids. Identification was based on presence of distinct features between the two groups of scales. Percid scales are ctenoid and have a dentate anterior margin, whereas cyprinid scales are cycloid (Davidson *et al.* 2003). Only one complete cyprinid scale was found through the whole of core SALG1, whilst fragments were more abundant for both of the fish groups. In zone III there was continued relatively strong representation of both types of scale, with the addition of pike *Esox lucius* (L.) scales also being found. Above zone III percid scale fossils became less abundant (both whole and fragments), whilst cyprinid scale fragments remained relatively constant throughout the zone.

Of the invertebrate remains presented in Figure 6.7, the head capsules of chironomid larvae were numerically dominant throughout the core, with a maximum of over 60 per g (DW) in the bottom marl section (zone IV). Zone IV was also where *Orthotrichia* sp. caddis cases, *Piscicola geometra* (L.)(fish leech) egg cocoons and oribatid mites had greatest abundances. The presence of *Piscicola geometra* cocoons suggests that submerged vegetation was present during this period, as this species eggs are often attached to plant stems (Odgaard and Rasmussen 2001). Above the marl section, in zone III, all invertebrate taxa appeared to decline in abundance, with the exception of remains tentatively identified as triclad (flatworm) egg cocoons. The abundance of trichopteran fronto-clypeus' (caddis fly larvae head shields), appears to be relatively constant through core SALG1, with occasional sporadic peaks. The taxonomic resolution to which several of these invertebrate groups could be taken makes ecological interpretation of the data uncertain. As many individual species are included in each group, e.g. the chironomids, bulked community density is subject to many disparate influences with shifts in individual

species abundances over time. However the types of organisms present can give a general impression of the past aquatic ecosystem structure and how it has varied over time.

Remains of cladocerans preserved in freshwater sediments include the ephippia, or dormant diploid stage. The Daphniidae are well represented in Figure 6.7, as this taxa has the largest ephippia. Smaller ephippia of chydorid species were observed in the finer fraction of the sieved sediment, but as the bodily remains of this group, such as head shields and carapaces, preserve well (Hann 1989), identification of these smaller ephippia was not attempted. The stratigraphy of cladoceran macrofossil remains shows that in the lower zones I and II, ephippia were very infrequent, with only the mud associated *Leydigia* genus represented throughout the section. A single ephippium of the plant-associated *Simocephalus* sp. was present at 79 cm and several more were found in zone II. At the top of zone II, and through zone III, *Daphnia hyalina* agg. and *Ceriodaphnia* sp. ephippia began to occur, with *Daphnia hyalina* agg. ephippia increasing steadily into zone IV, where these planktonic taxa were dominant.

Bryozoan statoblast species each had distinct profiles in abundance throughout core SALG1. *Plumatella* sp. were relatively infrequent in the lower zones I and II, increased through zone III and most abundant in zone IV. *Cristatella mucedo* (Cuvier) was most abundant in zone I and relatively infrequent through the rest of the core. *Lophopus crystallina* (Pall.) statoblasts were only found singly in four of the sediment layers analysed, with most occurring within zone IV.

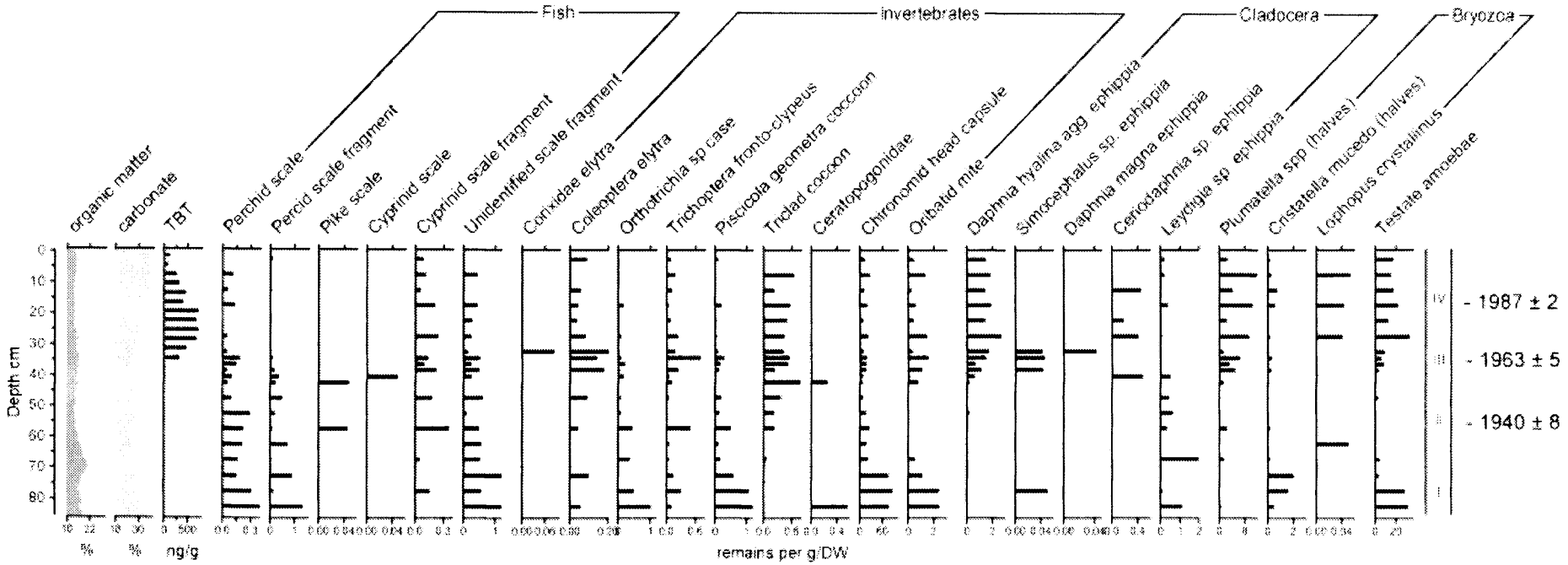

Figure 6.7 SALG1 vertebrate and invertebrate organism macrofossil stratigraphy.

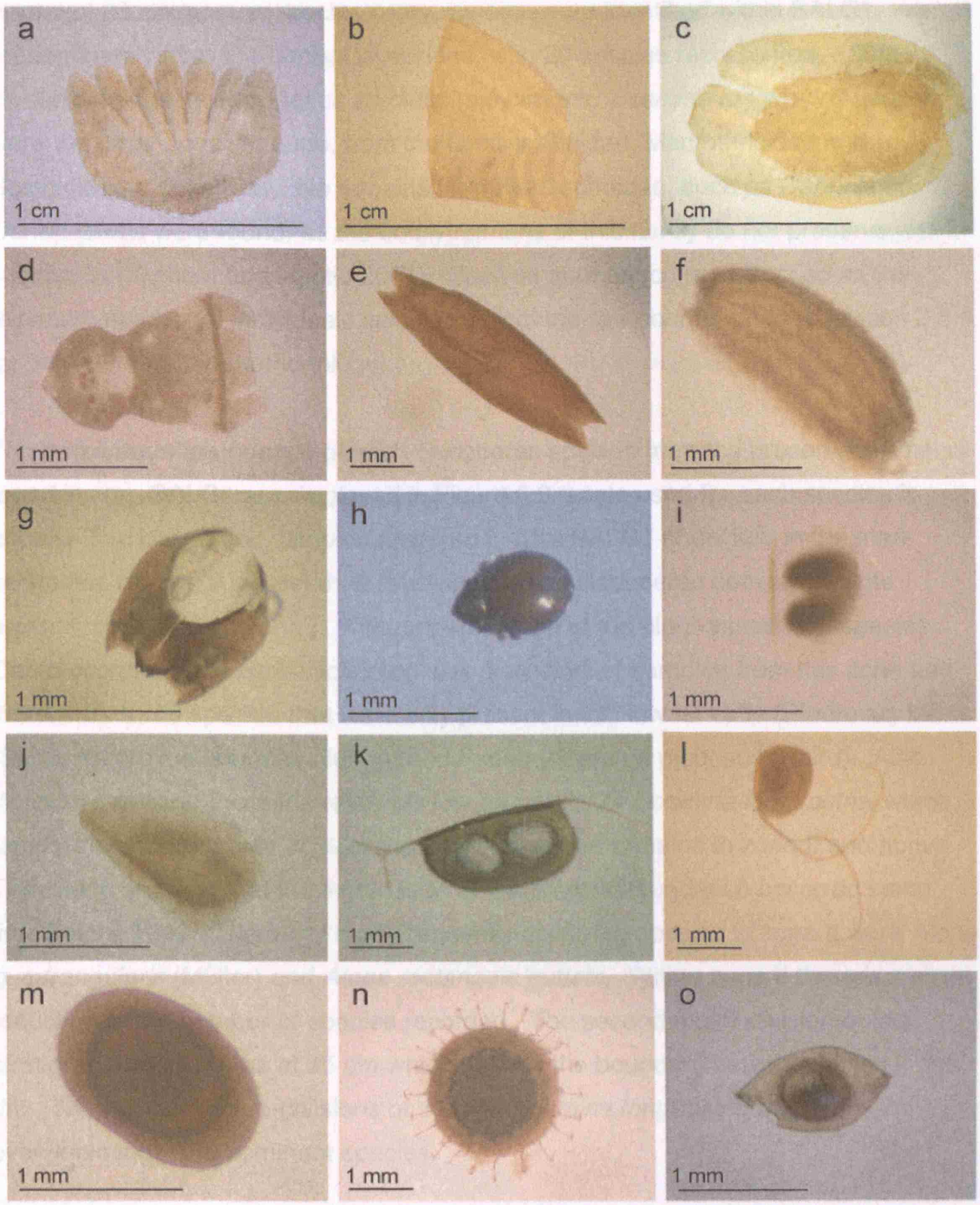

Figure 6.8 Examples of fish, invertebrate and bryozoan macrofossil remains found in River Bure broads sediment cores

(a) Percid scale; (b) Cyprinid scale fragment; (c) Pike scale; (d) Trichopteran fronto-clypeus; (e) Orthotrichia sp. caddis case; (f) Piscicla geometra egg cocoon; (g) Chironomid head capsule; (h) Oribatid mite; (i) *Daphnia hyalina* agg. ephippia; (j) Simocephalus sp. ephippia; (k) *Daphnia magna* ephippia; (l) Leydigia sp. ephippia; (m) Plumatella sp. statoblast; (n) *Cristatella mucedo* statoblast; (o) *Lophopus cristallinus* statoblast. (Photographs – D.J. Hoare)

A total of 23 cladoceran species proxy remains were identified within SALG1, predominantly from the family Chydoridae, with 20 species represented. *Sida crystallina* (Müller), *Ilyocryptus sordidus* (Liéven) and *Bosmina longirostris* (Müller) were the other species found, from the families Sididae, Macrothricidae and Bosminidae respectively. No remains from the Daphnidae, such as *Daphnia* or *Ceriodaphnia* were found, as the bodily remains of this family do not preserve well in sediments (Korhola and Rautio 2001). Species abundance is expressed as the minimum number of individuals calculated from the raw count data (see section 2.6.1 for the enumeration methodology).

The percentage abundance of each cladoceran species from the preserved remains found in core SALG1 are displayed in Figure 6.9 (scale used for each species is equal). The lowest two samples analysed in core SALG1 show that in the marl sediments below 70 cm depth, a relatively diverse cladoceran community was present, classified as zone I. A large percentage of the plant-associated species *Camptocercus rectirostris* (Schödler) was distinctive of samples from this zone and there were three species that were only present in this lowest zone (Pleuroxus laevis (Sars), Pleuroxus aduncus (Jurine) and Pseudochydorus globosus (Baird). Also distinctive of zone I was the relatively low proportion of *Bosmina longirostris*, which rapidly increased to over 40% of the total cladoceran remains in zone II and above. The dating suggest that the increase in *B. longirostris* abundance occurred some time before 1940. The other most frequently occurring species in zone II were *Alona quadrangularis* (Müller) and *Alona rectangula/guttata*. Within zone II there is a slight reduction in the number of species recorded. The second major division of this stratigraphical data was at 45 cm which defines the boundary between zones II and IIIb. Throughout all sub-divisions of zone III *Bosmina longirostris* was overwhelmingly the dominant species.

Figure 6.10 displays the same data expressed in absolute terms, in units of number of individuals per gram (on a dry weight basis). The scale bars in this figure are not equal between species, thus displaying the variation in relative abundance with depth more clearly for each taxa. In zone II, notable reductions in concentrations of both *C. rectirostris* and *Eurycercus lamellatus* (Müller) were observed. Both of these species are known to be strongly associated with the presence of submerged plants (Whiteside and Swindoll 1988). In the uppermost zone, zone III and all its sub-divisions, many species, especially those associated with submerged macrophytes

either disappeared altogether or had markedly reduced abundances e.g. *Graptoloberis testudinaria* (Fischer) and *Sida cristallina*. The final division of zone III, IIIc, shows a slight increase in concentration of *E. lamellatus*, perhaps suggesting a slight recovery after the most intense period of TBT contamination. The total concentration of cladoceran remains (far right of Figure 6.10) shows that after zone I ended the cladoceran productivity in Salhouse Broad increased rapidly. This pattern is largely driven by the large proportion of *B. longirostris* remains (Figure 6.9), but demonstrates the distinction between the heavily plant associated zone I and those subsequent. Overall, the classifications used for both concentration and relative abundance values, identified the same depth levels as break points between the distinct zones. There was only slight variation in the depths where sub-divisions within major zones were placed. In Figure 6.10 the boundary between zone IIIb and IIIa was coincident with the intitial detection of TBT dated as 1963.

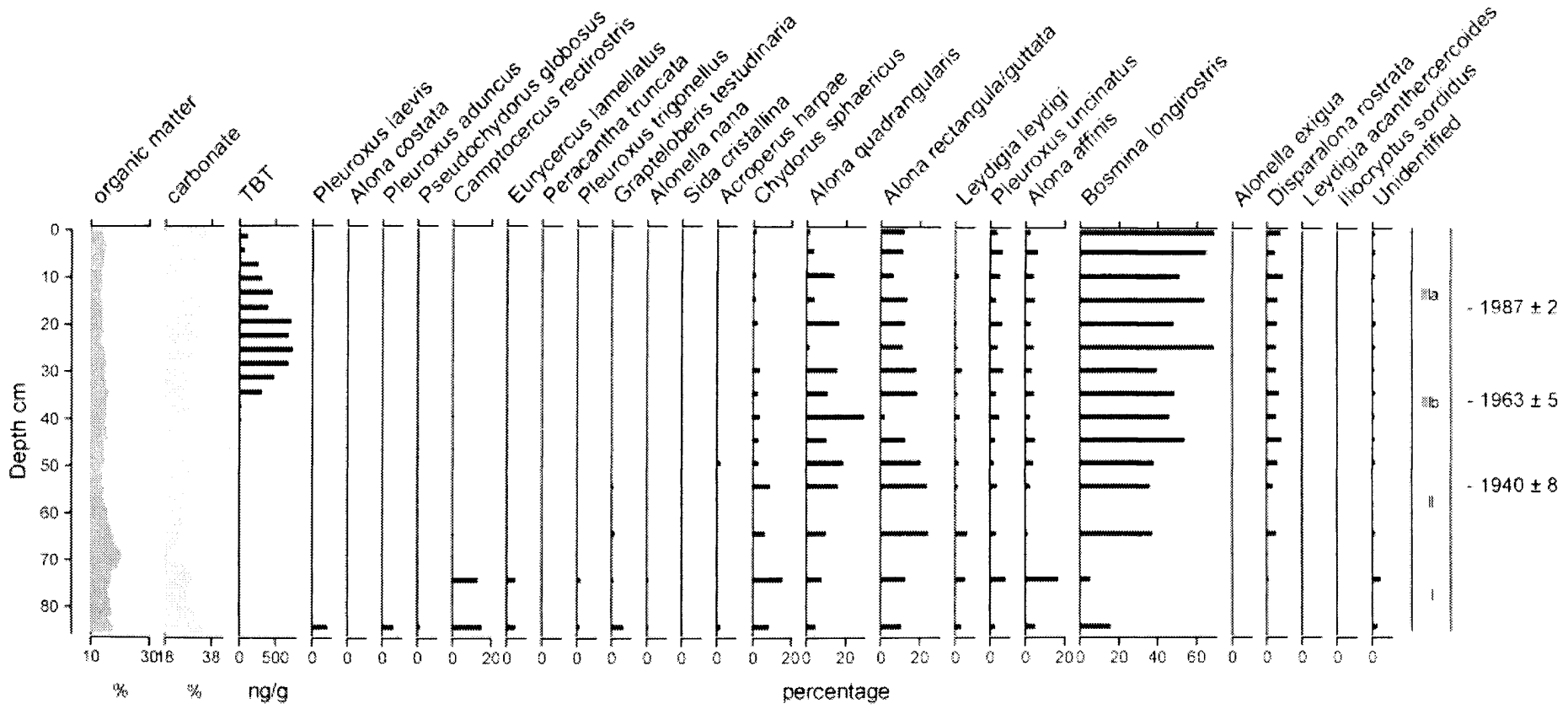

Figure 6.9 Percentage abundance of cladoceran remains in SALG1.

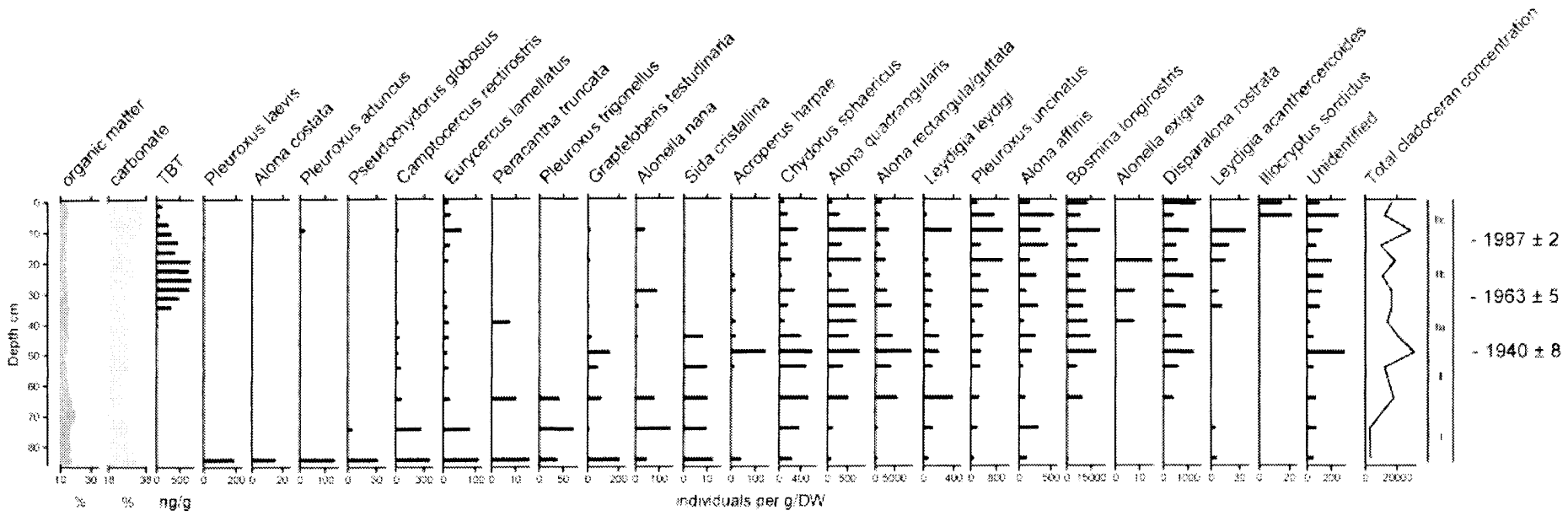

Figure 6.10 Concentration of cladoceran remains in SALG1.
(individual graph scales are not equal)

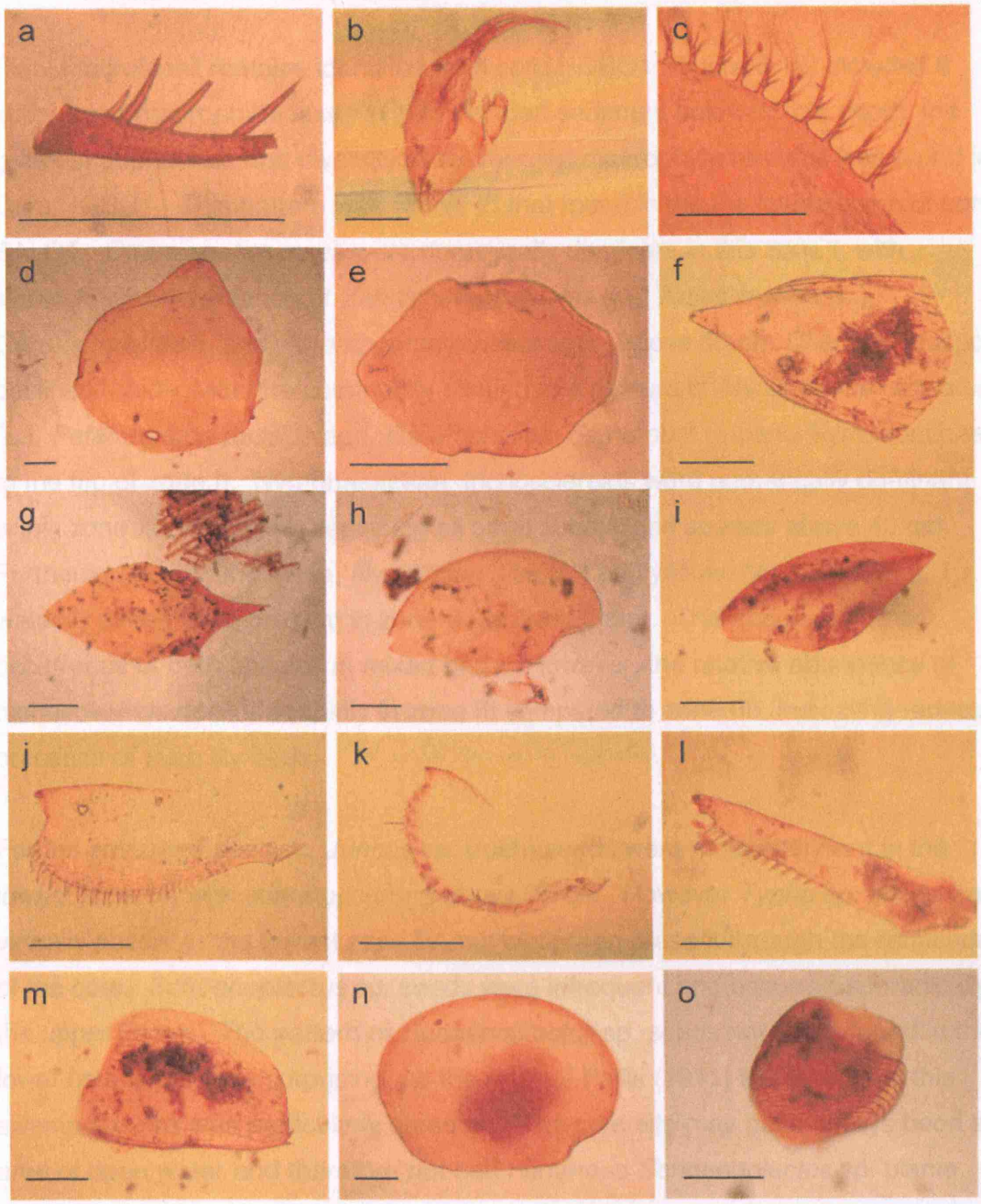

Figure 6.11 Examples of cladoceran subfossil remains found in River Bure broads sediment cores (Scale bars represent 100 µm).

(a) Sida cristallina anal claw fragment; (b) Bosmina longirostris headshield; (c) Ilyocriptus sordidus carapace setae; (d) Eurycercus lamellatus headshield; (e) Alona costata headshield; (f) Disparalona rostrata headshield; (g) Pleuroxus trigonellus headshield; (h) Acroperus harpae headshield; (i) Camptocercus rectirostris headshield; (j) Alona quadrangularis postabdomen; (k) Leydigia leydigi postabdomen; (l) Camptocercus rectirostris postabdomen; (m) Grapteloberis testudinaria carapace; (n) Pseudochydorus globosus carapace; (o) Alonella nana carapace.
(Photographs – D.J. Hoare)

6.1.2.2 Hoveton Great Broad – Bure Broads

Plant macrofossil remains identified from core HGBO1 (Figure 6.12) included 8 submerged macrophyte taxa. Within the marl sediment below 65 cm depth, the greatest abundance and diversity of submerged macrophyte remains was found in Core HGBO1. This pattern was similar to that found in the the lowest zone of core SALG1. *Chara* sp. oospores were numerically dominant in this zone I, with significant representation of *Zannichellia palustris* (L.), *Najas marina* (L.), *Ceratophyllum* sp. and *Potamogeton pusillus* agg. Above 65 cm *Chara* sp. oospores declined rapidly, with the community shifting to a mixture of *Myriophyllum spicatum* (L.), *Potamogeton pusillus* agg.,*Ceratophyllum demersum* (L.) and Nympaeaceae up to the top of zone II. Nymphaeaceae trichosclereids were numerically dominant within zone IIb, effectively replacing all other submerged species above 40 cm. Furthermore, both the white, *Nymphaea alba* (L.) and yellow, *Nuphar lutea* (L.) waterlily seeds were present in zone III (above 20 cm). This suggests the co-occurrence of both species in mixed beds. However, the relative abundance of trichosclereids declined rapidly in zone III compared to zone IIb, indicating reduced presence of such lily beds.

For the emergent species, *Juncus* sp. (rush) seeds were most abundant in the lowest zone IV, with some occurring above 35 cm. However *Typha* sp. seeds were virtually absent in the lowest zone IV, but were then present through the remainder of the core. *Schoenoplectus* sp. seeds were infrequent and occurred sporadically in the upper 25 cm. The pattern of *Schoenoplectus* sp. seeds not being found in the lower core is perhaps surprising, as the work of Pallis (1911) indicated that this swamp species was particularly common. The core site may have always been an area of open water and therefore not had numerous *Schoenoplectus* sp. plants nearby to leave seeds in the sediment record.

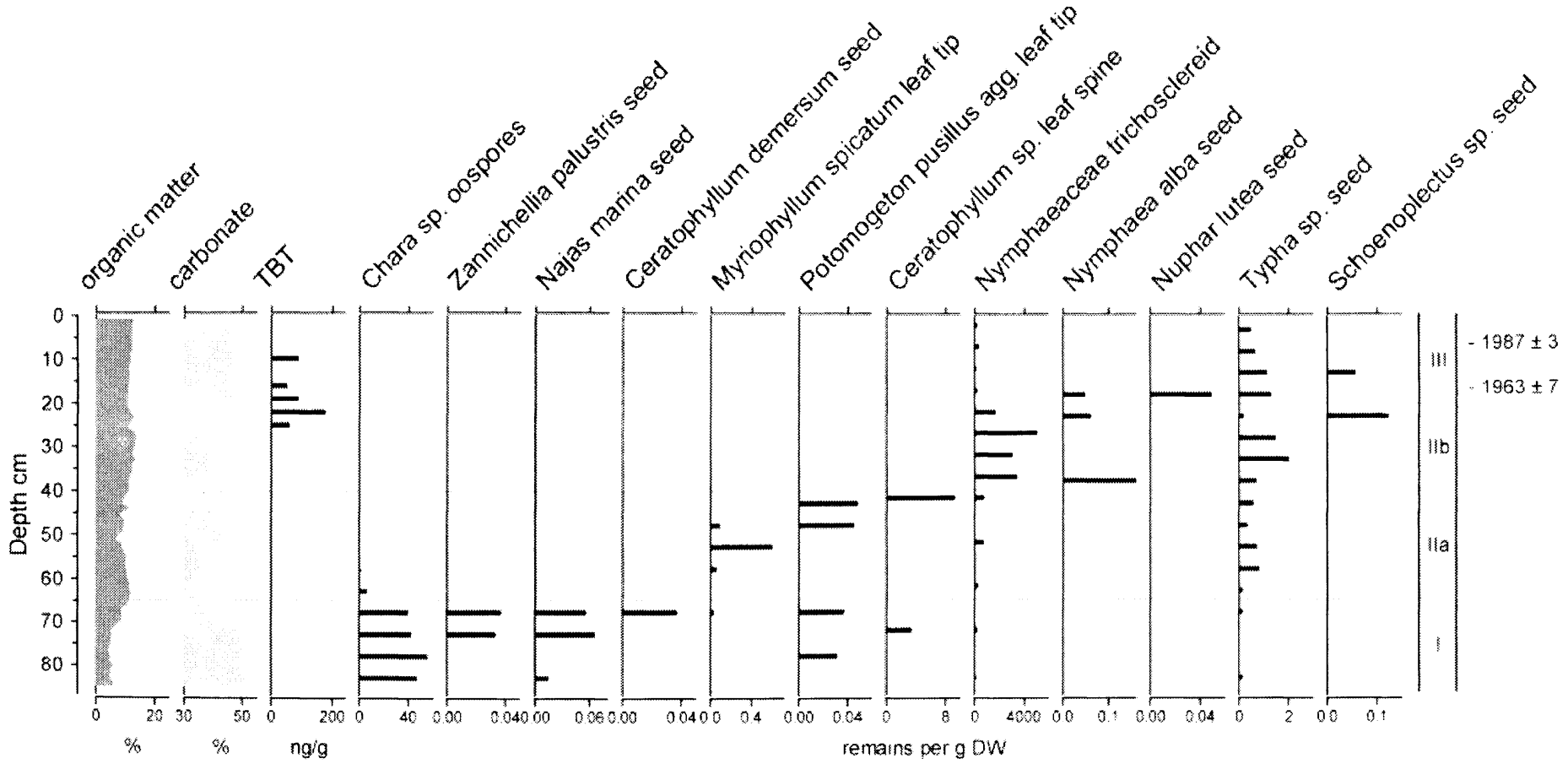

Figure 6.12 HGBO1 plant macrofossil stratigraphy.

The gastropod and bivalve macrofossil remains enumerated through core HGBO1 are given in Figure 6.13. At total of 17 taxa were identified with 13 species. All of the identified prosobranch gastropod taxa were recorded in the bottom, marl section of the core, below 65 cm, classified as zone I. The most abundant species were *Valvata cristata* (Müller), *Valvata piscinalis* (Müller), *Bithynia tentaculata* (L.) and *Lymnaea peregra* (Müller). The pulmonate gastropods were also well represented in this zone, as was *Pisidium* sp. (pea mussels). Above 65 cm mollusc diversity and abundance declined extremely rapidly, with most taxa absent throughout zone II, until around 45 cm where there was a limited recovery. Within zone III *Acroloxus lacustris* (L.) and *Gyraulus crista* (L.) appeared for the first time and juveniles of the genus *Valvata* were also found at densities similar to zone I. Zone III is characterised by large densities of bivalve glochidia (probably *Anodonta* sp. larval spats) and the appearance for the first time of *Dreisenna polymorpha* (Pallas). The most numerically abundant mollusc in the uppermost zone IV (above 25 cm), was *Gyraulus crista*, although it occurred at low numbers compared to the dominant taxa in zone I. *Acroloxus lacustris* declined and disappeared in this top zone. *Valvata piscinalis* and *Bithynia tentaculata*, which had both been found throughout zone III declined again within zone IV. *Valvata* sp. juveniles which were relatively abundant within zone II also declined above 25 cm.

The similar profiles of shell and operculum remains for the two *Bithynia* species suggest that either remain type could be used todetermine palaeoecological profiles for these species. The operculum remains were found in slightly greater densities compared to the whole shells suggesting better preservation in the sediment. Enumeration of the juvenile stages also provided a comparison of the temporal variation in deposition of the early life stage for each genus where juveniles were present. A high density of bivalve glochidia in zone IIa suggests either large recruitment, or possibly poor survival, during this period. This life stage of bivalves are obligate parasites on fish, so glochidia presence in sedimentary material is also indicative of host fish presence, including cyprinids and percids (Aldridge and Horne 1998).

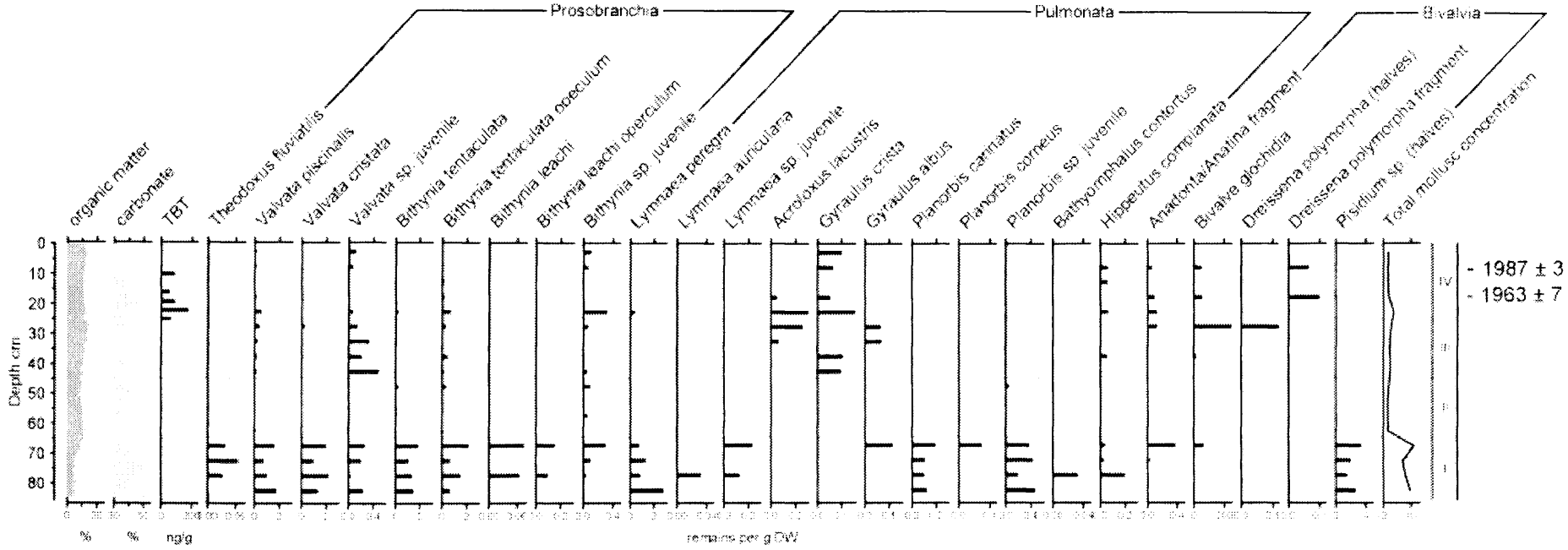

Figure 6.13 HGBO1 mollusc stratigraphy.

In comparison with SALG1, a similar range of vertebrate and invertebrate macrofossil remains were found within core HGBO1 (Figure 6.14). Fish scale remains were of similar taxa to SALG1, but exhibited different patterns of relative abundance over time. For example percid scales and fragments were represented in the lowermost zone I of HGBO1, though at a lower density than in SALG1. Cyprinid scales were infrequent and low in number within zone I. Percid and cyprinid scales both increased to a maximal concentration in zone III and then became less abundant in the uppermost zone IV, after the introduction of TBT. Sporadic single pike scales were found, so no inferences about the relative abundance of pike over time could be made. The most frequent macroinvertebrate remains found throughout the core were of Trichoptera, *Piscicola geometra* and Chironomidae. The latter had the greatest abundance in zone I. All invertebrate remain types appeared to have a reduced abundance in zone II. The various caddis fly remains, including *Orthotrichia* sp. cases and undistinguished fronto-clypeus parts, were all most abundant within zone III. However in the uppermost zone IV their abundance was much reduced, again occuring after the introduction of TBT. *Piscicola geometra* egg cocoon and triclad egg cocoon densities were also greatest in zone III and declined rapidly in zone IV, in a similar way to the caddis fly larval remains. Chironomid head capsules were common throughout the core especially in zone I. This family displayed only a slight reduction in density in the uppermost zone IV compared to zone III.

As in SALG1, cladoceran ephippia were virtually absent from bottom zone I. *Leydigia* sp. ephippia became dominant within zone II,, but declined rapidly in the lower half of zone III and subsequently became more frequent towards the top of this zone, at a similar time to the initial detection of TBT. In zone, above 55 cm, *Daphnia hyalina* agg and *Ceriodaphnia* sp ephippia also began to be detected sporadically. Ephippia of the genera *Daphnia*, *Ceriodaphnia* and *Leydigia* subsequently all had peak density in the uppermost zone IV. Of the bryozoans, *Cristatella mucedo* was most frequently found in the core. This species had a peak in abundance in bottom zone I and in zone III. In zone IV (above 20 cm), *Plumatella* sp. statoblasts became dominant, with a corresponding decline in *Cristatella mucedo*. The abundance of testate amoebae followed a similar pattern to *Orthotrichia* sp., *Piscicola geometra* and triclad egg cocoons, by peaking in zone III.

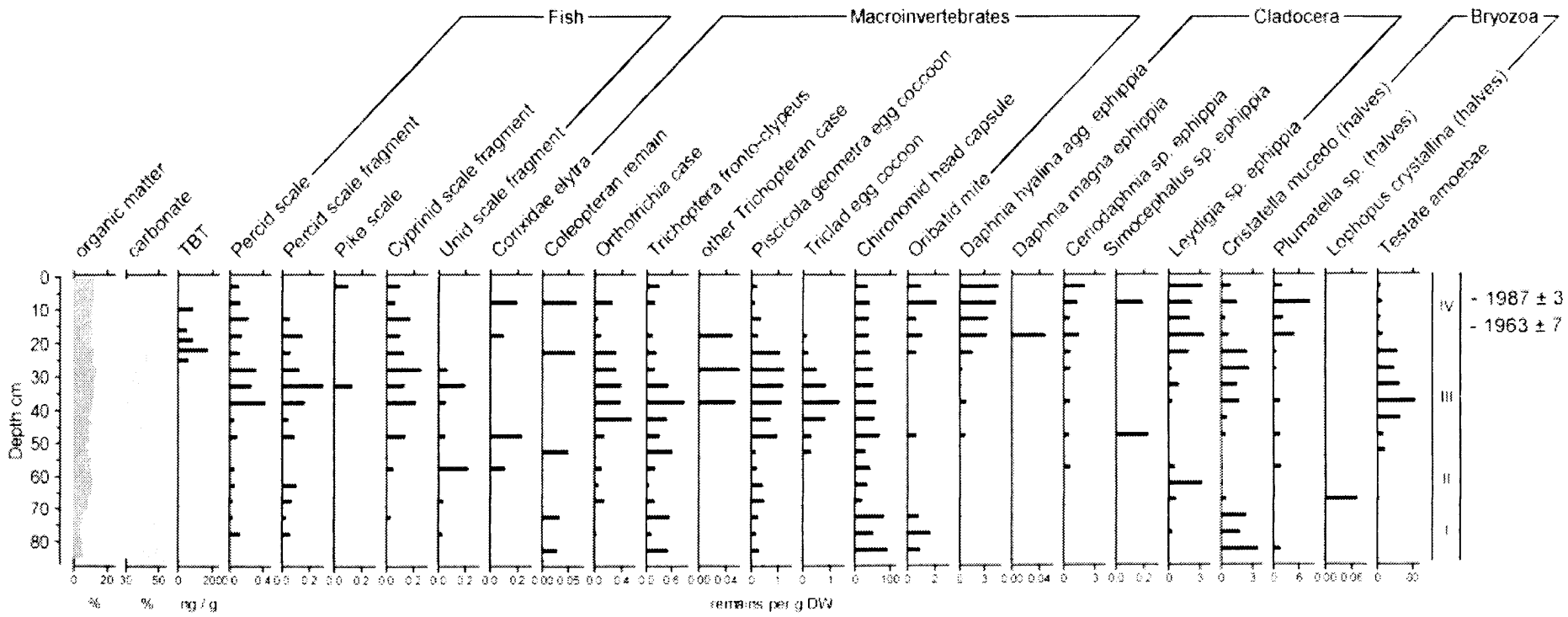

Figure 6.14 HGBO1 vertebrate and invertebrate organism macrofossil stratigraphy.

Within core HGBO1 remains of some 23 species of Cladocera were identified. results of the cladoceran remains was slightly different for the percentage and concentration data. This was due to the transformation of the count data to percentages which down-weighted the most dominant taxa. However, significant divisions at 65 and 24 cm were common to both data sets, which corresponds well with major units in the sediment lithostratigraphic data (Figure 6.1). The bottom zone I corresponds with the high carbonate marl sediments, zone II with the organic gyttja and zone III with the increased carbonate gyttja in the period following TBT contamination. At the bottom of the core, the lowest two samples were both dominated by*Camptocercus rectirostris*, *Chydorus sphaericus*, several *Alona* species and *B. longirostris* remains (Figure 6.15). The zonation indicates that these two samples were distinct from the rest of the core. Above 65 cm (zone II), the proportion of several species remains increased, including *Acroperus harpae* (Baird), *Pleuroxus uncinatus* (Baird) and *Alona quadrangularis* (Müller). The most abundant species remains between 65 cm and 24 cm were *Alona quadrangularis*, making up over 20% of the total, followed closely by *B. longirostris*. Above 24 cm (zone III), after the introdution of TBT, *B. longirostris*, *Alona rectangula/guttata* and *A. quadrangularis* became the most dominant remains.

Figure 6.16 shows that there was a relatively low density of cladoceran remains in zone I. Through zones II and III a stepped increase occured in the concentration of several species. Those species that peaked in zone III included *Peracantha truncata*, *Alonella nana*, *Acroperus harpae*, *Pleuroxus aduncus*, *Camptocercus rectirostris* and *Sida cristallina*. All of these species are particularly associated with submerged macrophytes (Whiteside and Swindoll 1988, Davidson 2006). The main change at the 24 cm zone division (the beginning of zone IV in Figure 6.16) was a dramatic reduction in the concentration of those plant-associated species which had peak concentrations in zone III. This pattern can be seen in the species graphs in Figure 6.16 between the *Peracantha truncata* and *Sida cristallina* profiles. All of these species displayed reduced concentration after the introduction of TBT. Species concentrations to the right of the diagram appeared to either not change or increase. Increase in density of remains in zone III and IV can be seen in the graph of total cladoceran concentration (far right of Figure 6.16). After the introduction of TBT maximal concentration of cladoceran remains occurred, again largely driven by *B. longirostris* remains, as in core SALG1.

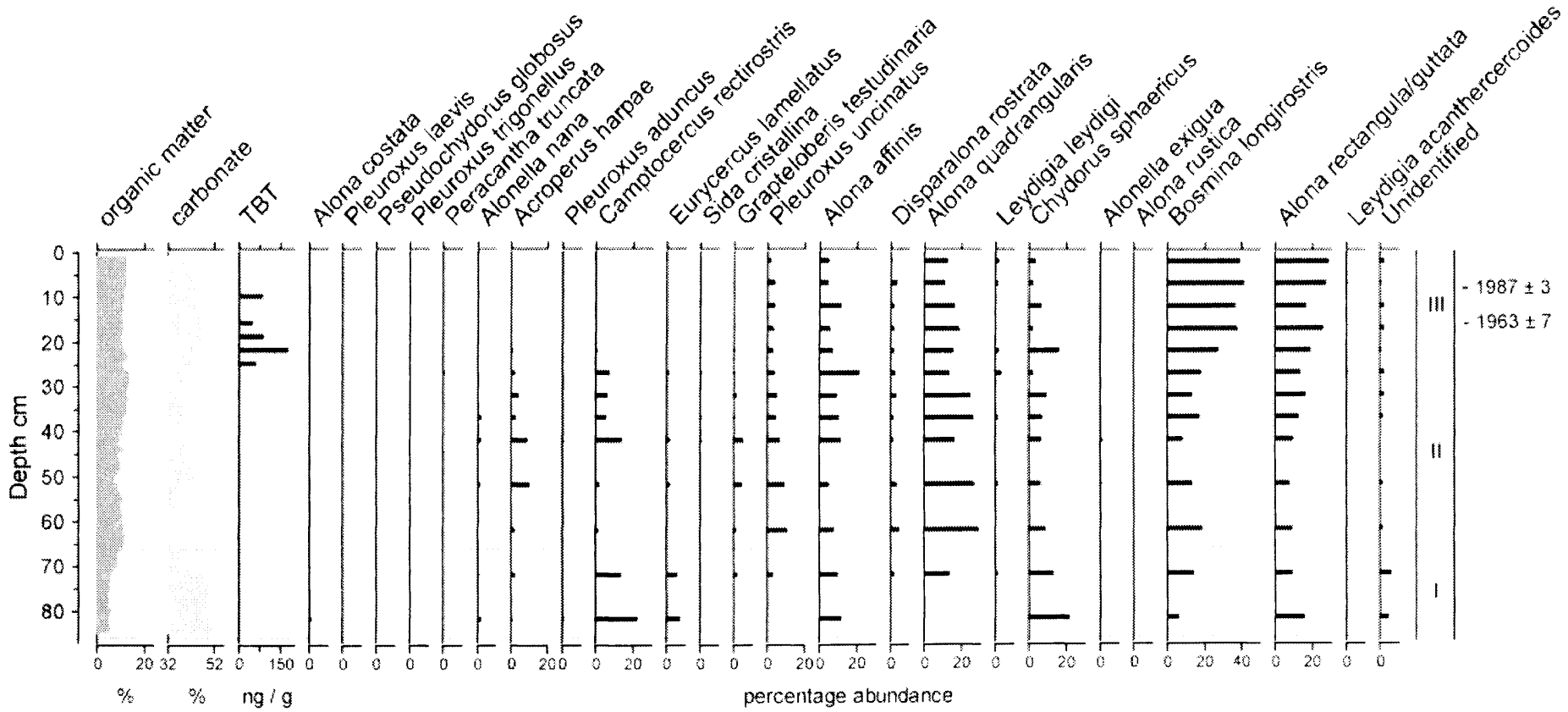

Figure 6.15 Percentage abundance of cladoceran remain types in core HGBO1.

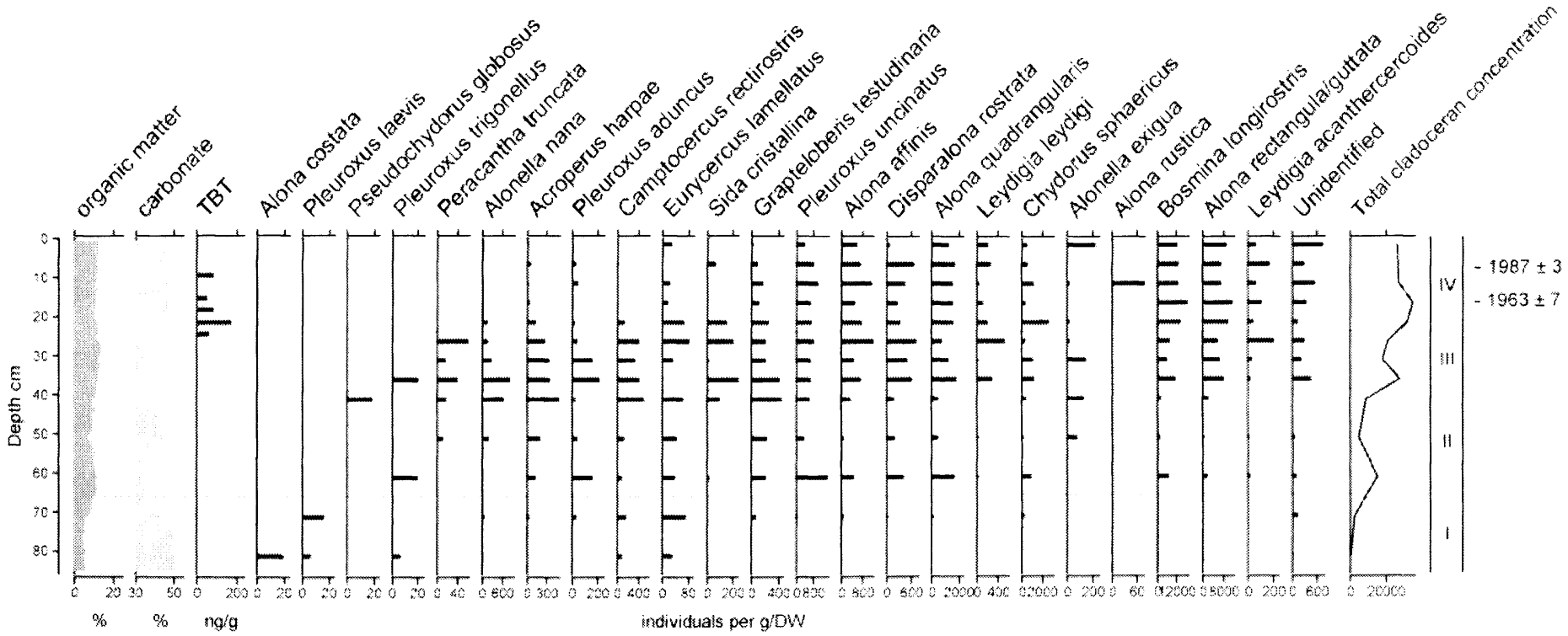

Figure 6.16 Concentration of cladoceran remains in core HGBO1.

6.1.3 Palaeoecological summary - Bure Broads

To summarise the palaeoecological changes observed in SALG1, Figure 6.17 shows the dominant taxa from each macrofossil group. Also shown are the geochemical results and the major changes in cladoceran community composition. The classification of cladoceran species as plant-associated or non-specific (largely mud-associated) followed (Whiteside 1970;Whiteside and Swindoll 1988, Davidson 2006). Plant-associated species identified by Davidson (2006) from mulitivariate analysis of chydorid remains in the surface sediments of a 39 shallow lake dataset included *Pleuroxus aduncus*, *Eurycercus lamellatus*, *Acroperus harpae* and *Pleuroxus laevis*. *B. longirostris* was the only truly planktonic species recorded from the analysis of preserved bodily remains. It appears that core SALG1 did not penetrate as far into the carbonate rich, charophyte oospore and mollusc dominated layer, as achieved in HGBO1, possibly due to a lower sediment accumulation rate at the latter core site. Below 70 cm depth, the sediment in SALG1 was characterised by a relatively high organic carbon and total nitrogen content, > 20% and > 2% respectively. In this lowest zone, plant associated cladocerans, mainly chydorid taxa, had good representation (up to 40%), as did plant associated macroinvertebrates. *C. sphaericus* was recorded separately in the community composition graph, as increases in this primarily plant-associated/littoral species have been shown not to necessarily indicate an increased abundance of macrophytes (Korhola 1999). Cladoceran Shannon diversity declined below the value of 2 above 70 cm and remained so through the remainder of SALG1.

Above 70 cm the marked decline in charophyte oospores and of several other proxies indicate that nutrient enrichment was having marked effects. For instance, the less negative organic $\delta^{13}C$ values above 70 cm depth (Figure 6.17), indicate a relative increase in lake productivity after this period (Schelske and Hodell 1995;Meyers and Lallier-Vergès 1999). The $\delta^{13}C$ values also show an increase at 40 cm depth, indicating a further increase in algal productivity. This inference of a switch having occurred at around 40 cm (~1960) is supported by the continued increase in proportion and concentration of remains of the planktonic cladocera *B. longirostris* (Figures 6.10) and a change in the composition of mollusc species remains (PCA axis 1 values) above this depth (Figure 6.17). *Daphnia* sp. and *Ceriodaphnia* sp. ephippia also begin a rapid increase in concentration above 40 cm, suggesting increased open water and abundant phytoplanktonic algae.

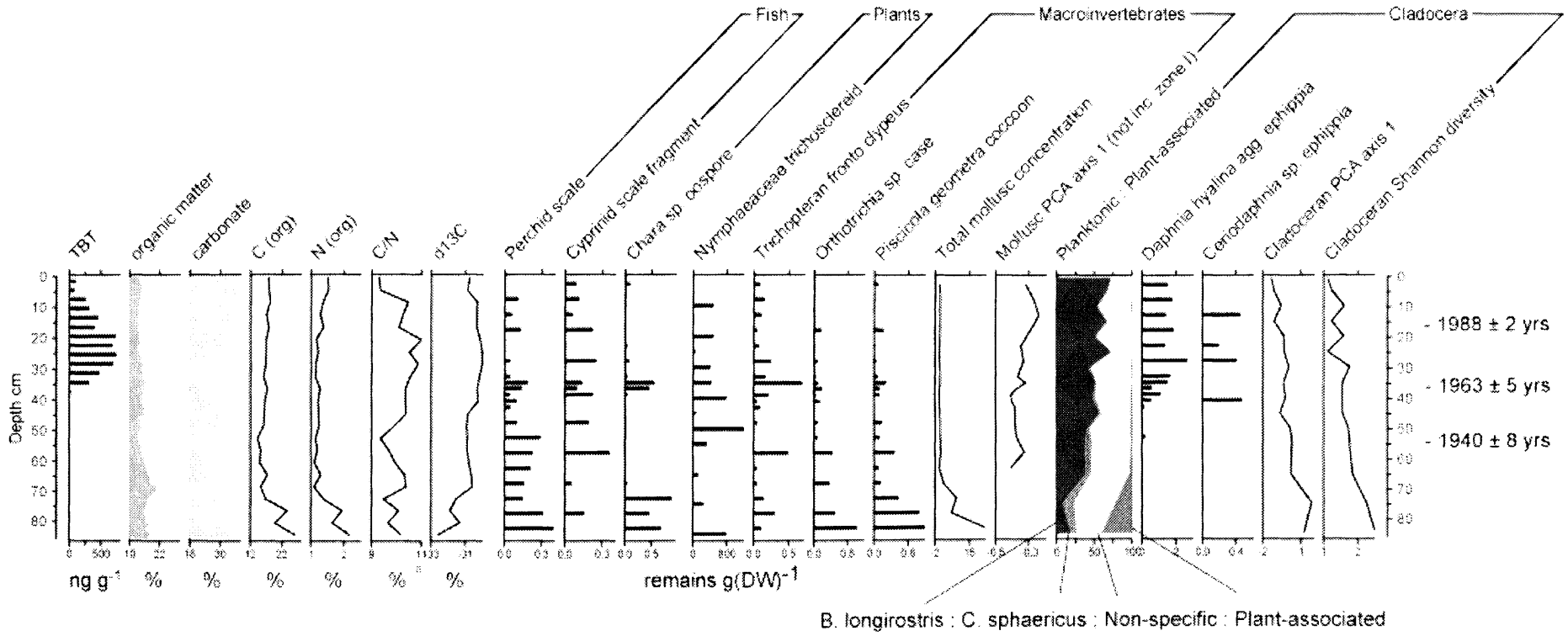

Figure 6.17 Summary of SALG1 palaeolimnological results

At the time of significant TBT detection in SALG1, at around 35 cm depth, some declines in taxa relative abundance were observed within the macroinvertebrate proxies. There was evidence of some species loss within the cladoceran community composition data (Figure 6.10), as may be expected from predictions of the elimination of the most sensitive species upon exposure to a toxic contaminant (Jak *et al.* 1998b;Hall *et al.* 2000;Leung *et al.* 2005a). Cladoceran species have been shown to be sensitive to TBT exposure, as highlighted in section 6.3. A slight decrease in average cladoceran Shannon diversity occurred above 30 cm (Figure 6.17). The relatively wide oscillations in species diversity during this period perhaps suggests an instability in the cladoceran community structure after TBT exposure. The daphnid species, as represented by the ephippial remains, were by contrast, most abundant during the peak period of TBT contamination, with numbers increasing prior to the initial detection of TBT. This observation may however be related to several abiotic and biotic factors, particularly a change to open water and an increased availability of the algal food resource (Whiteside and Swindoll 1988).

After the period of relatively high charophyte oospore and mollusc concentration ended in HGBO1 at around 70 cm (Figure 6.18), there were many indications of a continued presence of macrophytes and associated fauna. Several key differences between the two cores were apparent. Firstly, within the sediments above 70 cm in HGBO1, plant-associated cladoceran species persisted up core, before disappearing at around 20 cm depth. Also, the peak in abundance of plant-associated macroinvertebrate (excluding mollusc) remains occurred between 40 cm and 20 cm, a period characterised by a high abundance of waterlily remains. Trichosclereids had greatest concentration up to the early 1960s (according the the radiometric dating), which is also supported by the aerial photography of Hoveton Great Broad (Figure 2.9), showing extensive floating macrophyte growth in 1961, which had disappeared by 1969. These trichoschlereid leaf cells were present within SALG1 (max. 1000 g^{-1}), but never in the abundance recorded in HGBO1 (> 3000 g^{-1}). By contrast in HGBO1, *B. longirostris* did not assume dominance within the cladoceran community after the disappearance of charophytes as happened in SALG1 and the HGBO1 cladoceran Shannon diversity remained above 2 until around 25 cm. Previous research has shown that *B. longirostris* was the dominant planktonic cladoceran in the open water of Hoveton Great Broad during 1980 (Timms and Moss 1984). This report helps confirm that the palaeoecological record accurately reflects the historical cladoceran communities once present.

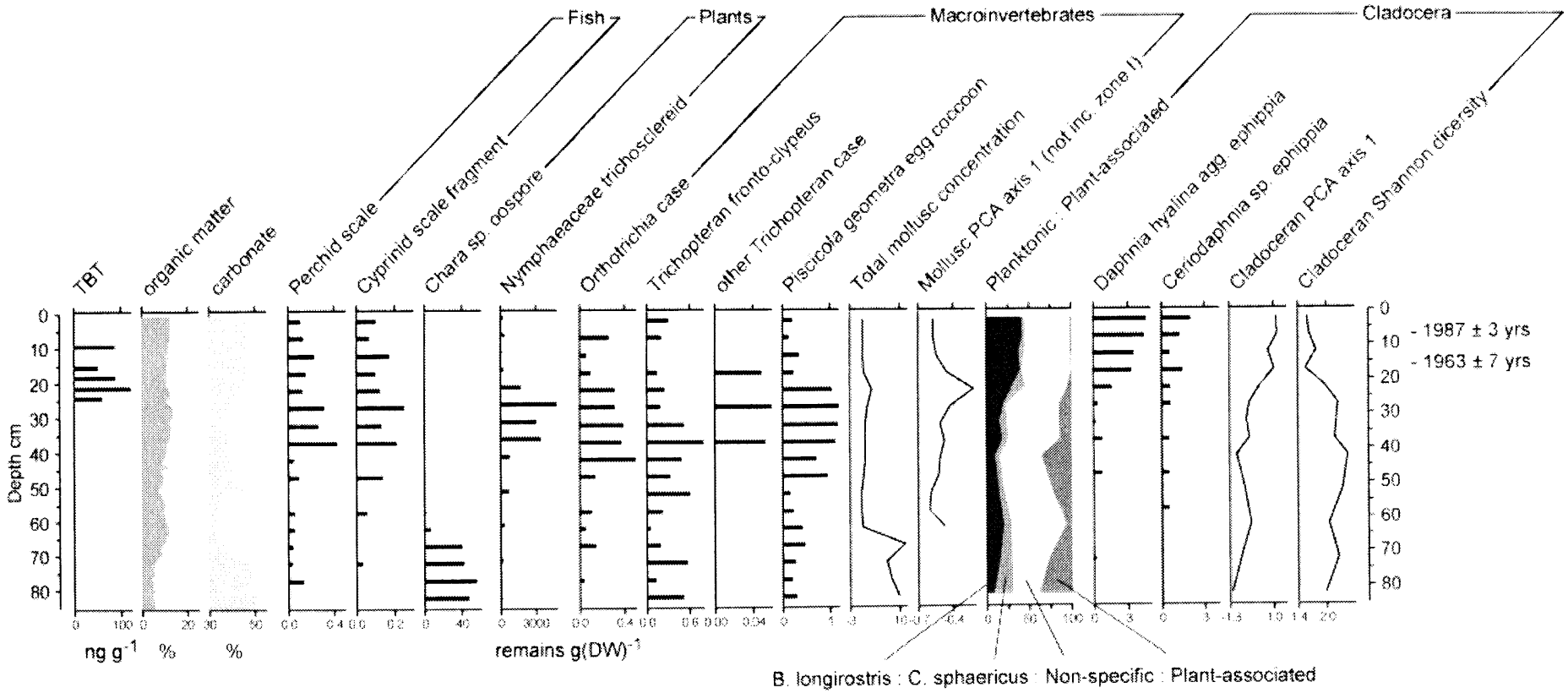

Figure 6.18 Summary of HGBO1 palaeolimnological proxy results.

Changes within the palaeoecological record of HGBO1, which occur in synchrony with the onset of initial TBT contamination at 25 cm are numerous and more distinct compared to SALG1. Both percid and cyprinid fossil scale abundances declined above 25 cm, the same time as the waterlily remains began to disappear (Figure 6.18). Above 25 cm the number of caddis fly remains decreased, as did the abundance of *P. geometra* cocoons. Total mollusc concentration appears to have changed little above 70 cm, as the relatively high values around 10 individuals per gram (D.W.) masked subsequent changes in the profile. However, the PCA axis 1 scores show that at 25 cm a distinct event in the composition of the mollusc remains occurred. Another significant ecological change observed at 25 cm was a community switch within the Cladocera, from a mixed fauna with presence of plant-associated taxa, to a community dominated by planktonic taxa. *B. longirostris* which had been present in an even proportion in the lower core, increased above 25 cm to become the dominant remain type. This point in the core also saw a sudden and rapid increase in *D. hyalina* agg. and *Ceriodaphnia* sp. ephippia remains. Both the summary statistics derived from the cladoceran analysis displayed on the right-hand side of Figure 6.18 shows distinct change above 25 cm. No such similar shifts in these metrics were observed lower in the core and are interpreted as reflecting a switch from plant-associated to planktonic taxa and also an overall decrease in cladoceran (mainly chydorid) species diversity. These changes in HGBO1 are synchronous with the introduction of TBT in the core profile.

6.2 Hickling Broad – Thurne Broads

Figure 6.19 displays the variation in the flux of macrofossil remains through core HICK1 from Hickling Broad. Calculation of remains in flux units (remains cm^{-2} $year^{-1}$) was made possible within this core, as the radiometric chronology was of a higher resolution compared to the Bure broads cores (section 5.4.1). The TBT profile indicated that contamination did not begin until 12 cm depth therefore no macrofossils were analysed below 26 cm. The total depth of lake sediments was much lower than in the cores obtained from the River Bure broads, with the deposition rate in HICK1 calculated to have been continually low, only reaching a rate of 0.5 cm yr^{-1} during the last decade. A lesser total number of macrofossil taxa were found in this core compared with cores SALG1 and HGBO1.

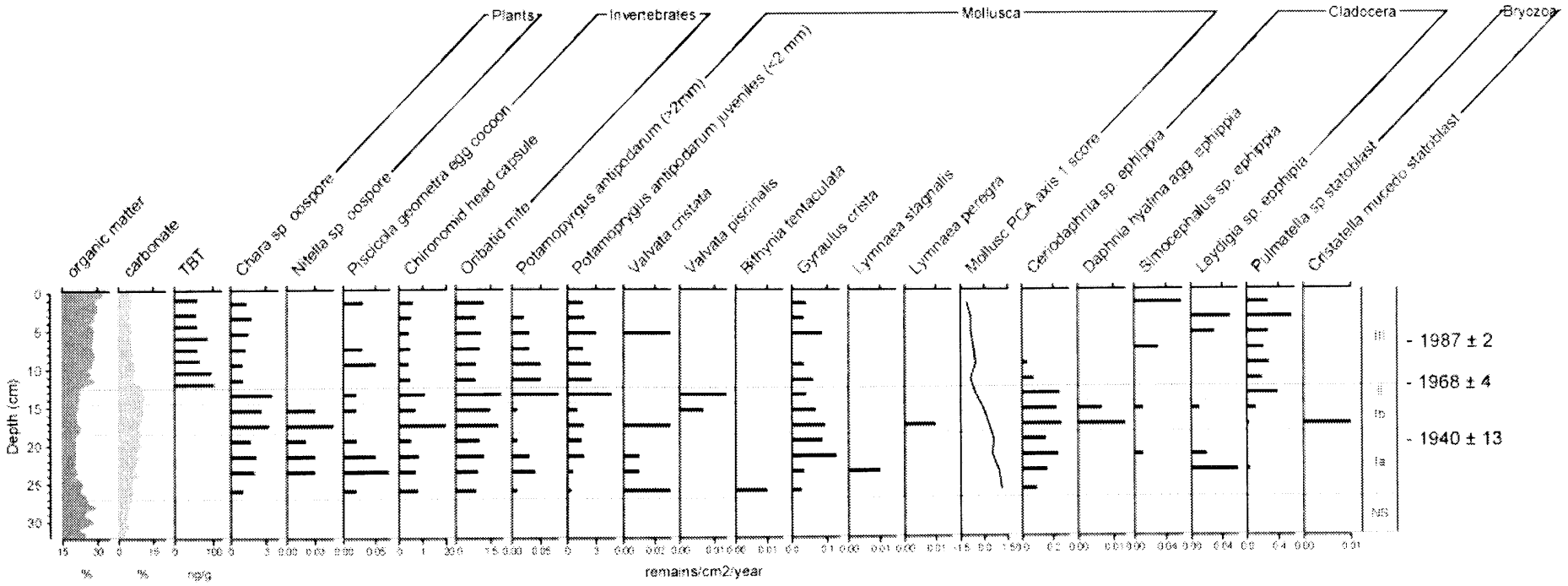

Figure 6.19 Macrofossil remains from core HICK1
(NS – not sampled)

One explanation for the lower number of remain types in HICK1 may be the smaller volume of sedimentary material available for macrofossil analysis at each depth level. The average volume of wet sediment sieved for macrofossil analysis from HICK1 was only 16 ml per level compared to over 60 ml for the River Bure broads cores, collected with the wide-diameter corer. Hickling Broad is also a brackish lake, which may have reduced the total number of taxa present through exclusion of saline intolerant species, such as within the mollusca and Cladocera. It has also historically been a charophyte dominated lake, which will have acted to exclude other macrophyte species. However, despite the reduced number of taxa present, zonation of macrofossil results from core HICK1 display clear temporal changes in the remains (Figure 6.19).

The lowest zones Ia and Ib in Figure 6.19 were characterised by abundant *Chara* sp. oospores and also frequent detection of *Nitella* sp. oospores, dated as being present in the early 1900's through to the 1960's. The dated decline in oospore aundance in HICK1 in the late 1960's (Figure 6.19) suggests that the charophyte dominated phase persisted until much more recently in Hickling Broad that in the Bure broads. Phillips (1963) provides direct evidence of charophytes in Hickling Broad into the early 1960s. Of the seven mollusc species found through this core, all were found within these two lower zones. The most abundant species was the mud snail *Potamoprygus antipodarum*, which was found mostly as immature individuals < 2 mm. However, the depth profiles of adult and immature stages of this species followed a similar pattern throughout the core, suggesting a continuously high reproduction rate, but with relatively few individuals reaching the adult stage. *Gyraulus crista* was also found frequently, with greatest numbers occurring in zone Ia. Ephippia of four cladoceran taxa were also found in this lower part of the core. The most abundant ephippia were of *Ceriodaphnia* sp. which were found continuously through zones Ia and Ib. In the lower zones bryozoan statoblasts were also found sporadically and in low numbers.

Zone II appears to represent a transition in abundances of the HICK1 macrofossil remains dated as ocurring in the late 1960's. *Chara* sp. oospores progressively increase up core and reach their maximum at 14 cm, as do oribatid mites and *Ceriodaphnia* sp. ephippia. These three proxies however decline sharply above 12 cm (1968 ± 4 years), with *Ceriodaphnia* sp. completely disappearing from the core in zone III. *Nitella* sp. oospores were absent from zone II and above. Conversely,

Potamopyrgus antipodarum numbers suddenly increased at 14 cm, as did *Plumatella* sp. statoblasts. The peak abundance of these two proxies was greatest within zone III. The only other mollusc species present relatively frequently in zone III was *Gyraulus crista*, but in lower numbers than in the bottom section of the core. In zone I, the cladocerans *Simocephalus* sp. and *Leydigia* sp. were only found sporadically and had never been common lower down in the core. *Plumatella* sp statoblasts persisted through zone I to the top of the core.

The division in the biostratigraphic data between zones II and III occured at the same depth as the proportion of carbonate suddenly decreased at 13 cm. The carbonate profile matches most closely that of the *Chara* sp. oospores, suggesting charophytes have been a dominant source of carbonate to the sediment within Hickling Broad. The mollusc PCA profile suggests that this community was changing gradually over time, up until around 12 cm depth, after which a relatively stable period of low total abundance and diversity ensued. The first detection of TBT (and DBT) in the core was at 12 cm depth. This occurs at the same time as the sharp decline in the abundance of charophyte oospores and daphnid ephippia.

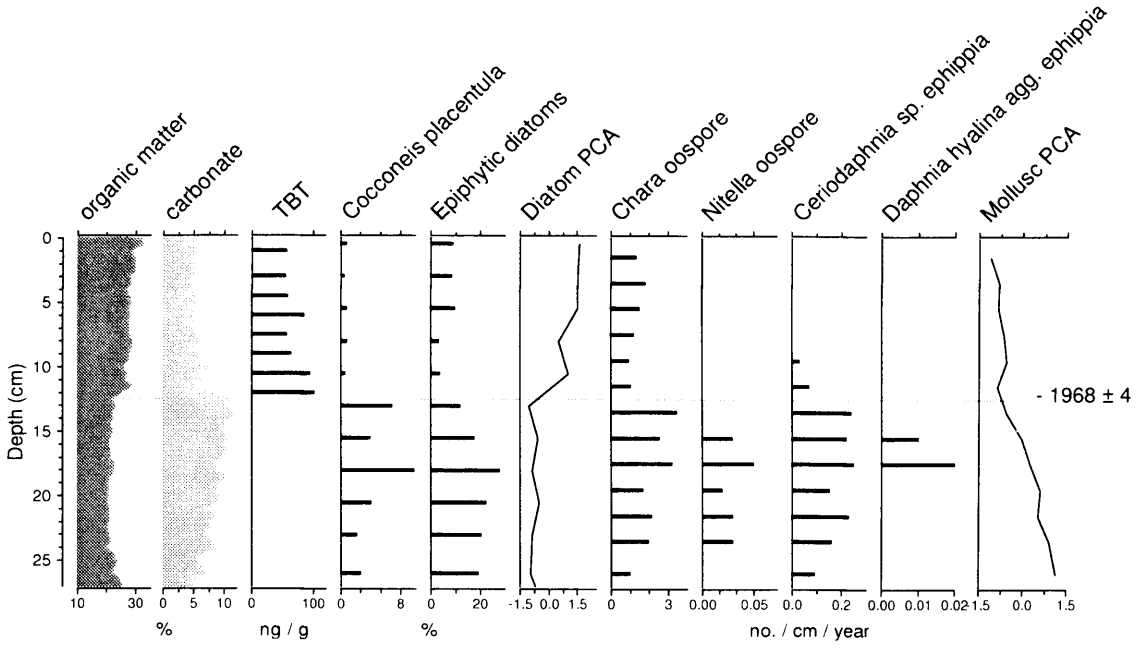

Figure 6.20 Summary of HICK1 palaeolimnological results

From the HICK1 diatom data presented in Sayer *et al* (2006) it is clear that the greatest change occurred at the same time as first detection of TBT within the core (Figure 6.20). Species that declined most were those strongly-associated with plants, such *Cocconeis placentula* and other epiphytes. The decline in these taxa closely follows the decrease in charophyte abundance, as inferred from the number of oospore remains.

6.3 Toxicity of TBT to freshwater organisms

To determine the sensitivity of freshwater aquatic organisms to exposure to TBT, the USEPA database ECOTOX was queried (see section 1.3.5.3 for details). All of the toxicity tests within this database were conducted with TBT in solution. As the present study has focussed upon the distribution of TBT preserved within sediment, the historical dissolved TBT data will act as a guide as to the likely exposure levels at the time of active usage. Table 6.1 gives the maximum reported concentrations of dissolved TBT from the River Bure waterway (sites in order of distance downstream).

Table 6.1 Maximum reported dissolved TBT concentrations from the River Bure waterway.

Date	Location	TBT (ng l^{-1})	Reference
1986	River Bure - Coltishall	77	MAFF (1993)
July 1989	River Bure - Wroxham	491	Waite *et al* (1989)
July 1989	Landamores boatyard	1620	MAFF (1993)
July 1986	Wroxham Broad	898	Waite *et al* (1989)
Sept 1986	River Bure, Horning	276	Waite *et al* (1989)

To summarise the ecotoxicological data, only the most sensitive end-points that demonstrated an adverse reaction to dissolved TBT at 2000 ng l^{-1} or less are presented in Appendix 9.6. All of these tests displayed a negative impact upon the test organism of one or more of the Darwinian fitness traits of growth, reproduction or survival. References for individual test results cited in this section are given in Appendix 9.6.

The lowest recorded effect of TBT on any organism was the reduction in growth of the green alga *Scenedesmus quadricauda* at 16 ng l^{-1} in a 12 day static water test. At higher concentrations (up to 2000 ng l^{-1}), negative effects on insect larvae;

molluscs (gastropods and bivalves); oligochaete worms; fish; cladocera; crustaceans; and amphibians have been consistently observed. Of particular interest in the present study are the impacts of TBT exposure upon algal grazing invertebrates which have been shown to be effective in controlling both epiphytic (Jones and Sayer 2003) and planktonic (Stephen *et al.* 2004b) algal populations in shallow lakes. Depletion of grazer populations, and thus reduction in the functional role of these organisms, is in this case hypothesised as a perturbation within shallow lakes, that can indirectly promote algal dominance, through removal of a key feedback mechanism which acts to maintain macrophyte growth. Algal grazing taxa that have shown particular sensitivity to TBT exposure include gastropods, with the species *Biomphalaria glabrata* suffering high mortality (LC_{60}) after 85 days exposure at 100 ng l^{-1} and *Lymnaea stagnalis* had reduced reproduction after 33 days at 320 ng l^{-1}. Of the insects, mayflies *Hexagenia* sp. exposed for 21 days were found to have reduced growth (LOEC) at 900 ng l^{-1} and an acute response (LC_{50}) at 1200 ng l^{-1}. Phytoplanktonic grazers were mainly represented in the toxicity tests by the ubiquitous test species *Daphnia magna*, which showed negative effects on reproduction (LOEC) after 21 days of TBT exposure at 400 ng l^{-1}, with acute responses occurring after 14 days at 1000 ng l^{-1}.

As well as algal grazers, sediment dwelling organisms were also amongst the most sensitive taxa. These included the non-biting midge larvae *Chironomus plumosus* which had the second most sensitive end-point measured, with an acute LC_{50} response at 50 ng l^{-1} after 96 hours. Similarly tubificid worms had increased mortality (LC_{50}) at 100 ng l^{-1} after 96 hours. Swan mussel (Anodonta cygnea) growth was reduced at 200 ng l^{-1} after an 8 day exposure. Sediment dwellers such as *Chironomus plumosus* are functionally important within shallow lakes as their burrowing activity has been shown to increase oxygen diffusion within the surface sediment layers (Polerecky, Volkenborn, and Stief 2006), thus stimulating microbial activity and nutrient recycling (Hansen, Mouridsen, and Kristensen 1998). Perhaps more significantly however, benthic-dwelling filter feeders such as non-biting midge larvae and bivalves can also contribute significantly to the clearance of phytoplanktonic algae from the water column (Polerecky *et al.* 2006;Maguire and Grey 2006).

Negative impacts of TBT exposure upon fish has also been observed at sub parts per billion concentrations. Rainbow trout *Oncorhynchus mykiss*, a commonly used

fish test species experienced increased mortality (LC_{25}) at 200 ng l^{-1} after 110 days; the guppy *Poecilia reticulata* had reduced growth at 320 ng l^{-1} after 91 days; and the minnow *Phoxinus phoxinus* suffered mortality at 820 ng l^{-1} after 9 days. Fish were generally less sensitive than the invertebrates, but percid species are reported as more sensitive than cyprinids (Martin *et al.* 1989).

Overall, it is clear that at the TBT concentrations reported to have been present in the River Bure waterway, the impact upon freshwater organisms would have been widespread. Relatively high dissolved TBT concentrations were maintained throughout the summer months (June-September) in navigable areas (Waite *et al.* 1989). This would have extended the exposure time of organisms present, enhancing the possibility of longer-term chronic effects (Fent 2003). Whilst laboratory based toxicity tests have limitations when extrapolated to effects observable in the field, a comparative study between the sensitivity of ecotoxicological endpoints to TBT in these contrasting test environments found them to be generally similar and within the same range (Selck *et al.* 2002). Figure 6.20 shows the pathway of TBT contamination in freshwaters and a simplified aquatic food web that would be exposed.

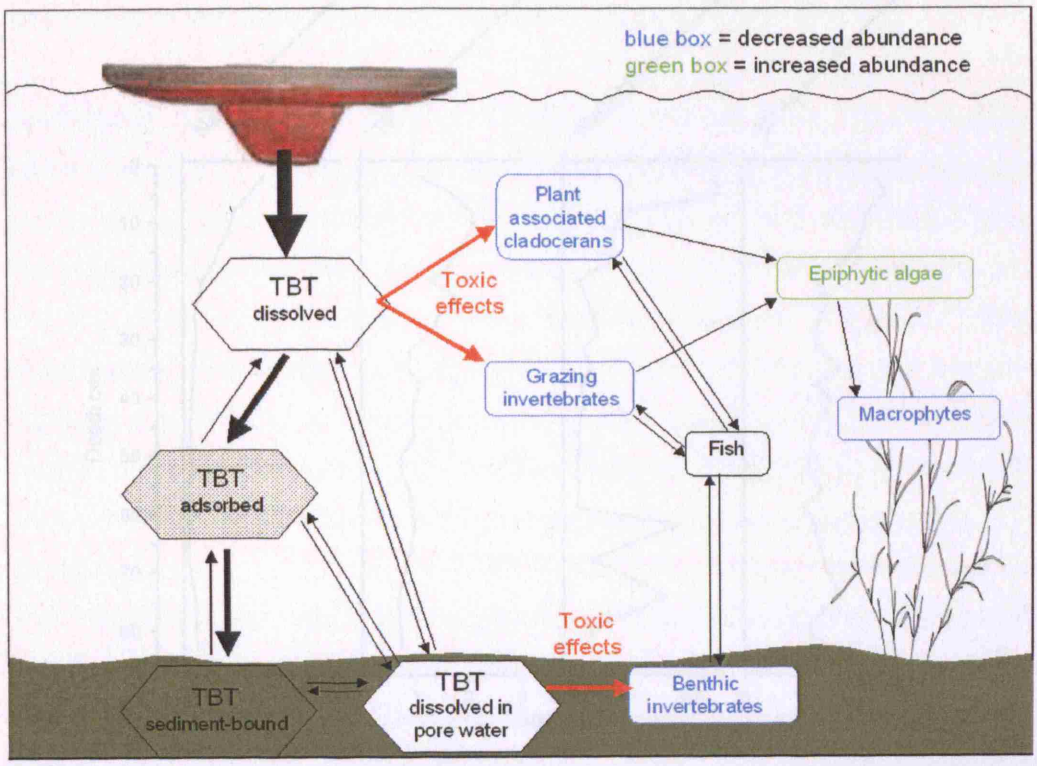

Figure 6.20 Major pathways of TBT contamination from boat to sediment, showing a simplified aquatic food web at risk from TBT exposure.

6.3.1 Evidence of progressive eutrophication

As detailed in section 1.3.2, there are numerous human-induced chemical stresses which can act upon the structure and function of aquatic ecosystems. During the recent period represented by the short sediment cores (post 1900) collected in the present study, increases in phosphorus input to the shallow lakes of the Broads has been observed (Moss 2001). Sediment Total Phosphorus and NaOH-extractable P were quantified in both the SALG1 and HGBO1 cores (Figure 6.21). In the present study the mobile P fraction has been quanitified within the Bure broads cores using an NaOH extraction method, as outlined in (Ruban *et al.* 1999). NaOH-P as defined by this method consisted of both inorganic-P, loosely adsorbed to sediment exchange sites and that bound to Al, Fe and Mn oxides and hydroxides. Both the NaOH-P and TP results display strong concentration increases with decreasing depth within both SALG1 and HGBO1 cores. TP concentrations were of a similar range to those previously reported for River Bure broads sediments (Pitt *et al.* 1997). The peaks of NaOH-P below 60 cm in HGBO1 are unexplained and their significance cannot be determined from the few data presented here.

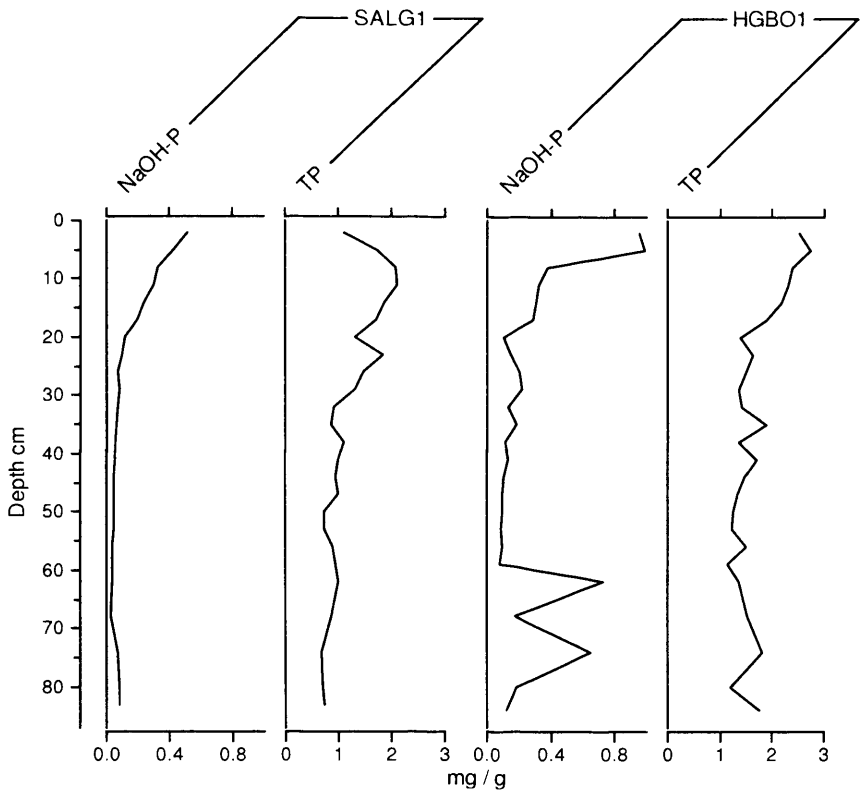

Figure 6.21 Sedimentary phosphorus stratigraphy of Bure broads cores.

Palaeolimnological studies on shallow lakes in Florida, USA, have interpreted variation in sedimentary P concentration as directly representing variation in historic limnetic P concentrations (Brenner *et al.* 1999b;Kenney *et al.* 2002). However, strong diagenetic effects upon the vertical distribution of labile, or loosely-bound, inorganic-P within sediments profiles have been demonstrated (Penn *et al.* 1995;Rydin 2000;Gao *et al.* 2005).

The observed pattern of decreased P concentration with depth has been suggested to represent high concentrations of temporarily stored, organic-bound P in the surface sediments, which is gradually released to the overlying water, leaving relatively P depleted sediments at depth (Søndergaard, Windolf, and Jeppesen 1996). Increases in limnetic P concentration have clearly occurred in the Broads (Johnes, Moss, and Phillips 1996;Moss 2001), but the validity of direct inference from sediment P profiles is questionable, hence the development of indirect models such as diatom-inferred P transfer functions (Bennion 1994;Battarbee *et al.* 2001). This technique was however not adopted in the present study due to the limitations in shallow lakes as highlighted by the work of Bennion *et al* (2001). The large proportion of diatoms in the genus *Fragilaria*, common in Broads cores, have been found to mask or increase the predicted historic TP levels, due to the insensitivity of this genus to variation in limnetic TP concentrations (Sayer 2001).

Additionally, the increases in TP and NaOH-P observed at the top of the Bure broads cores (Figure 6.21) seem to occur after the biological proxy evidence suggests that planktonic production became dominant in these lakes. The sediment P results of the present study therefore do not appear to effectively trace the increase in limnetic P that is known to have occurred in the River Bure over the time period under investigation. Expression of P deposition as flux would enable better evaluation of changes over time, which would be possible through obtaining sediment dating at greater depths in the cores. Figure 6.22a displays changes in sedimentary P concentrations relative to major shifts in palaeoecological remains in SALG1 (zonation based on cladoceran results, Figure 6.9). No significant change in NaOH-P or TP profiles were observed in zone IIa when plant-associated cladocerans disappeared and concentration of *Daphnia hyalina* agg. ephippia increased. This change in the cladoceran community structure indicates that Salhouse Broad had experienced a previous decline in submerged plant vegetation prior to the 1963 date and the introduction of TBT. Macrofossil evidence suggests that Salhouse Broad

had beds of waterlilies and some other sparse macrophyte growth (Figure 6.4) prior to TBT contamination. The profile of $\delta^{13}C_{(org)}$ in SALG1 also suggests that a shift in lake production occurred at 40 cm depth, as inferred by the the step change in the $\delta^{13}C$ values at this time. Directly inferring variation in NaOH-P and TP concentrations in the core as a proxy for limnetic P clearly has limitations and other data suggest this method is not reliable.

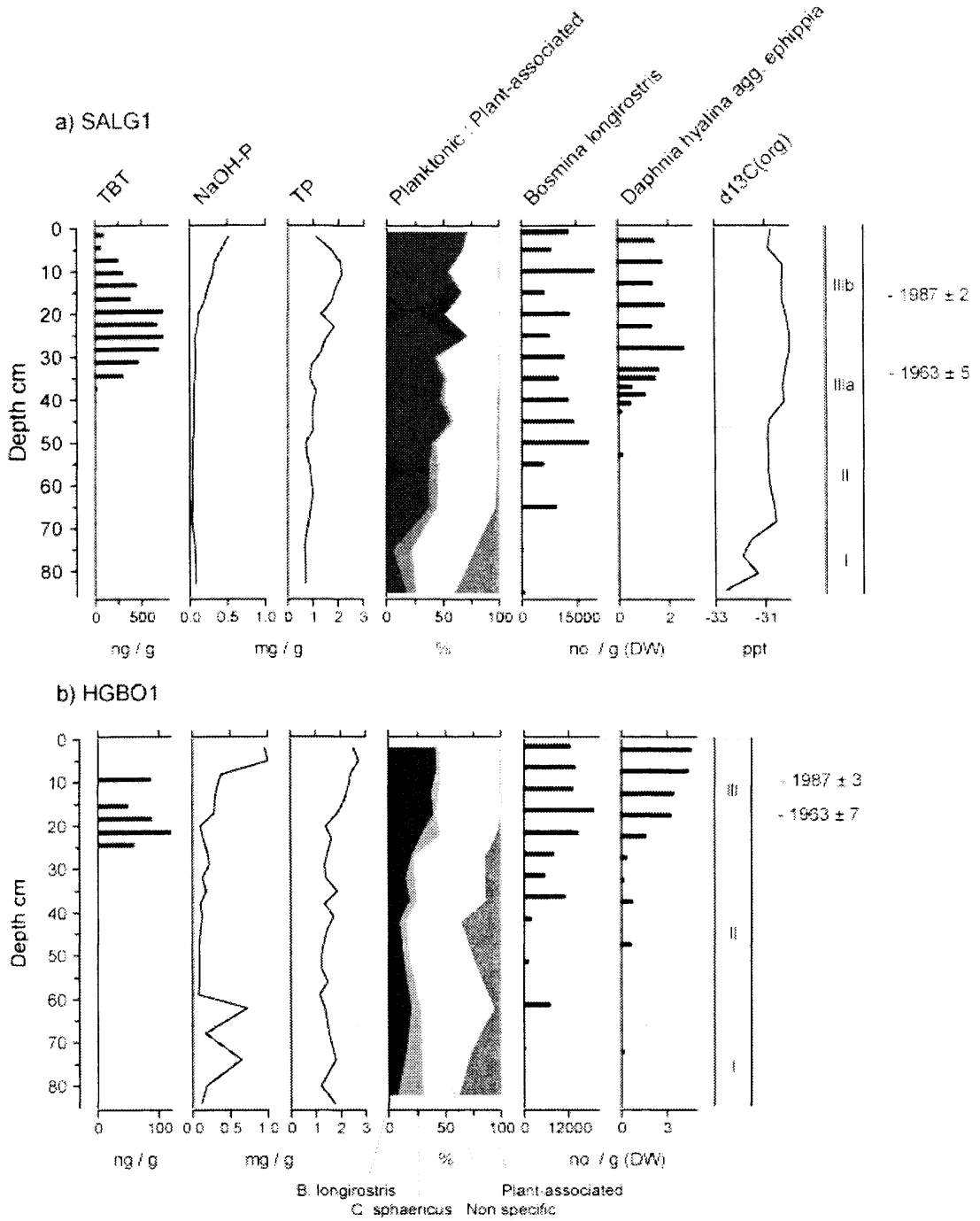

Figure 6.22 Sedimentary P concentrations relative to palaeoecological change in a) SALG1 and b) HGBO1.

Within Hoveton Great Broad (Figure 6.21b), the increase in sedimentary P concentrations was observed shortly after the loss of plant-associated claodocerans and the increase in *B. longirostris* and *D. hyalina*. These changes in biological proxies were however coincident with the initial detection of TBT, with P increases apparently occurring afterwards. The variation in relative abundance and submerged macrophyte species present and the associated fauna between these two broads indicates that they responded differently to the stresses exerted upon them.

6.4 General discussion

Macrofossil analysis from broads sediment cores has revealed that Salhouse, Hoveton Great and Hickling Broads were all characterised by predisturbance conditions where charophytes were significant components of the macrophyte community. Associated with this charophyte period were highly abundant and diverse mollusc communities and the presence of several plant-associated cladoceran species. After this period, the trajectory of ecosystem deveopment in each lake appears to have been markedly different. In the lowest layers of SALG1 there was a mixed macrophyte community, with species indicative of some nutrient enrichment such as *Myriophyllum spicatum* and *Zannichellia palustris* alongside the charophytes. Within SALG1, a rapid decline in charophytes and most of the other macrophyte species occurred well before TBT was first detected. This sudden decline in charophytes was also observed at a similar sediment depth in HGBO1. At the same time as the charophyte oospores declined rapidly in SALG1 and HGBO1, as did the molluscs. After the major decline in charophyte oospore abundance in HGBO1 however, there was evidence for a diverse macrophyte community up until first detection of TBT. This is supported by the aerial photography in Figure 2.9 which shows abundant submerged macrophytes in Hoveton Great Broad in 1946 and 1961. In both SALG1 and HGBO1 waterlily remains were common plant macrofossil prior to the introduction of TBT, but in HGBO1 these rapidly declined with the onset of TBT contamination. Sparse plant macrofossils were found in the sediments after the introduction of TBT, suggesting very little macrophyte growth. Aerial photography (Figure 2.9) shows minimal plant growth in 1969 and 1980 and previous surveys have confirmed the depauperate macrophyte community present in Hoveton Great Broad during the period of active TBT usage (Morgan 1972;Jackson 1978).

Plant macrofossils were not particularly abundant above 70 cm in the cores from the Bure broads, so the results presented can only provide an indication of the macrophyte species present. However, the relatively rich sub-fossil data from the aquatic organisms is supportive of the interpretations given. The major change observed in the SALG1 and HGBO1 palaeoecological data, coincident with the introduction of TBT, is a shift in cladoceran community structure, especially for HGBO1.

Within SALG1 the effects of nutrient enrichment upon the cladoceran community was evident prior to the introduction of TBT. After the major decline in charophytes, the increase in *Bosmina longirostris* and the decline in plant-associated cladoceran taxa suggests that the subsequent macrophyte community within Salhouse Broad was not extensive or stable, prior to TBT contamination. However, other macrofossil evidence suggests that a full switch to dominance of planktonic production did not occur until much later, when *Daphnia hyalina* agg. ephippia began to rapidly increase in the early 1960s (Figure 6.17), suggesting open water and abundant algal populations. In this case detection of low/no abundance of direct macrophyte proxies may fail to show persistence of plants when other more abundant proxies indicate otherwise. What is clear is the lack of any recovery towards the top of the core.

In HGBO1 the extent and stability of the macrophytes present after the post-charophyte crash appeared to be greater than that in SALG1. Most noticable was the persistence of molluscan and insect grazers and plant-associated cladocerans much further up the core compared to SALG1. The mollusc component of the macroinvertebrate grazing community in both SALG1 and HGBO1 displayed changes in community structure at a similar depth to the first detection of TBT contamination. In HGBO1 a rapid shift to more planktonic cladoceran taxa occurs after TBT is first detected, exemplified by the increase in *D. hyalina* agg. and *Ceriodaphnia* sp. ephippia. The largest shift in cladoceran PCA axis 1 score and decrease in overall chydoroid species diversity also occurs in synchrony with TBT introduction. There is a loss of the insect grazers and a reduction in the abundance of percid scales and waterlily remains at the onset of TBT detection. This suggests a catastrophic shift in aquatic ecosystem structure that occurred within an environment with elevated nutrient concentrations. The role of TBT cannot be clearly defined

through these results, but does highlight the synchronicity of the contamination and the ecological shift.

The difference in the ecological histories of these two adjacent broads is striking, given that the River Bure water supplies each. It seems that their submerged ecological structure was markedly different, particularly during the period after charophyte abundance initially declined. Both sites subsequently assumed greater ecological similarity after TBT contamination began, when fully planktonic production was operative. Hoveton Great Broad therefore appears have been more resilient to the effects of nutrient enrichment than Salhouse Broad, as it maintained more features of a macrophyte dominated shallow lake. The cause of the earlier degradation of Salhouse compared to Hoveton Great Broad can only be guessed at, but could possibly have been due to greater water exchange delivering a higher nutrient or suspended solid load from the river and/or greater disturbance from boat traffic, given that it is a navagable broad. Core WROX2 collected from Wroxham Broad had no macrophyte remains in it, suggesting that the navigable Bure broads have historically been depauperate in submerged plant growth away from the margins (Sayer *et al* 2006).

Within HICK1 a charophyte dominated macrophyte community was prevalent up until around the same time as TBT was first detected in the core profile (c. 1969). This date for the decline in charophytes is supported by observations at the time (see references cited in Appendix 1 of Bales *et al.* (1993)). As in the River Bure waterway, Hickling Broad suffered from increased nutrient enrichment, especially so in the late 1960s, and also increased salinity from increased efficiency of coastal land drainage pumps (Moss 1978). These potential causes of stress will all have exerted an influence upon the macrophyte community in Hickling causing a loss of ecological resilience making the system more vulnerable, as demonstrated by the experimental work of Barker *et al.* (2007). Their work showed that at increased salinity levels, the plant dominated state, in mesocosms aimed at replicating the sediment and water conditions of Hickling Broad, became unstable. Declines in plant biomass and species richness was observed through the increased release of phosphorus from the sediments and increased algal turbidity. However, the present study also indicates that the presence of TBT is an additional factor that closely matched the timing of the switch which occurred in the late 1960s.

6.4.1 Cause and effect in palaeoecological reconstructions

Previous palaeoecological reconstructions using zooplankton and benthic cladocera as indicators of toxicant impacts have demonstrated wider ecosystem changes, such as within fish and algal communities (Miskimmin *et al.* 1995;Manca and Comoli 1995). These previous studies have benefited from *in-situ* ecological monitoring during the pollution event, which has assisted interpretation of subsequent palaeoenvironmental results. Cause and effect coupling in the present study is complicated by the fact that some faunal species reductions that occurred after TBT exposure, may have resulted from the loss of the submerged plant habitat. For example, did the loss of plant-associated cladocerans occur before or after loss of the plant architecture upon which they depend. The results of the macrofossil and cladoceran analyses represent a record of the effects of the several influences acting to shape the aquatic ecological structure, rather than a sequential record of clear cause and effect relationships of TBT alone. However, the general cladoceran community impoverishment observed in HGBO1, and to some extent SALG1, after the introduction of TBT, is supported by similar patterns observed by (Yan *et al.* 1996;Kerfoot, Robbins, and Weider 1999). In both of these studies, the cladoceran community never fully recovered after the initial toxic event, both as a result of continued toxicity, lack of recruitment and food-web changes that prevented return to pre-disturbance conditions once the toxicity had abated. Furthermore, in a study of toxaphene dosing in two lakes of contrasting trophic status (Miskimmin *et al* 1995), variation in the response of cladoceran communities between the two lakes was evident, as for Salhouse and Hoveton Great Broads.

Ascription of causality in palaeolimnological studies of toxic impact is assisted where the ecological changes are very clear and the pollutant profile can be shown to have represented lethal exposure from bioavailable levels of the toxicant (Miskimmin *et al.* 1995;Manca and Comoli 1995;Ilyashuk *et al.* 2003). Determination of how bioavailable the TBT was prior to deposition in the sediments of the lakes presented has not been possible, so represents an unknown factor in ascribing causality through toxicological effects. However, not all pollution profiles in palaeolimnological studies are directly related to observed ecological shifts. For example, Paterson *et al.* (2003) found that PCB contamination in sediment cores was not related to any algal or chrysophyte change as the sedimentary concentrations would not have been of a sufficient level to induce an ecotoxicological response at the time of PCB

release to the lakes. Stansfield *et al.* (1989) found clear ecological changes but the pesticide profiles generated displayed high variability that prevented firm conclusions as to the timing of initial and maximal exposure. These studies highlight the difficulties in ascribing cause and effect relationships from such data. The spatio-temporal determination of TBT distribution within the study area and its potential ecotoxicological risk has helped reduce some of these methodological uncertainties in the present study.

6.5 Conclusions

The contrasting palaeolimnological results from Salhouse and Hoveton Great Broads demonstrates that the condition of the lakes prior to the first detection of TBT has influenced the degree to which the lakes could have been further degraded by toxicological effects from TBT. Relatively little change in macrophyte remains was observed in Salhouse Broad at this time, as a shift to a less macrophyte rich condition had already occurred. However, some observable ecological changes at the onset of TBT contamination included changes in the zooplankton community structure, and reduced abundances of some macroinvertebrates. Increased abundance of *D. hyalina* agg. ephippia was suggestive of a large shift from benthic to planktonic production at this time. However, the increase in this taxa was against a background of apparently increasing toxicological stress form rising TBT usage. How much influence TBT exerted on organisms is clearly difficult to determine from such retrospective studies. The neighbouring Hoveton Great Broad, which had maintained a submerged macrophyte community, albeit one influenced by an increased nutrient load, displayed a more clear shift to planktonic production at the same time as the onset of TBT usage. The ecotoxicological influence of TBT on sensitive taxa is a potential impact that therefore could have contributed to the observed shift.

The pattern of macrophyte loss and associated fauna and micro-flora within Hickling Broad has been shown to be similar to that observed in Hoveton Great Broad. The sensitivity of the macrophyte dominated state clearly being highlighted by the rapid ecological switch observed, which was coincident with the first detection of TBT in these cores. These conclusions are based on multiple lines of palaeolimnological evidence, generated through mulit-proxy analysis, to reveal the historical patterns of this proposed toxicant-mediated lake degradation.

Collection of cores from other broads associated with the River Bure, but which retain some macrophyte growth, would have strengthened assumptions as to the drivers of ecological change. For example Salhouse Little Broad and Hudson's Bay (both connected, but not navigable) currently and have historically had dense growth of waterlilies (Timms & Moss 1984; Broads Authority 2004). Lack of knowledge of how these sites have apparently maintained at least waterlily populations through the TBT contaminated period would add significantly to understanding of how TBT interacts with the biota. Analysis of a core from a broad that experienced loss of macrophytes but which had no exposure to TBT would have also acted a kind of control site, which would have shown how nutrient increases and factors other than TBT can precipitate plant loss. A further factor that would increase the confidence in the actual timing of changes in the paleaolimnological records from TBT exposed broads would have been replication of analyses within core and replication through mulitple cores from individual broads. For example the work of Moss (1988) demonstrates the variablility in sediment composition down core from several cores collected within Hoveton Great Broad. However, the increased volume of sediment analysed for the macrofossil analysis in the present study goes some way to increase the representitvity of a single core. Also the results from cores WROX2, SALG1 and HGBO1, all collected with a 5 km stretch of the River Bure valley wetland may be seen as a form of replication at the river scale. Simple statistical analysis would have also helped to back up certain statements as to relationships between variables within the cores.

In shallow lake systems, not only are potential ecotoxicological changes masked by the complexity of trophic interactions between organisms, but obtaining an accurate record of these events from proxy evidence contained in the sedimentary record is itself a scientific challenge. However, what the multi-proxy approach does provide is several, often independent lines of evidence that demonstrate not only changes in particular species abundances over time, but also fundamental changes in ecosystem structure. As such, multi-proxy palaeolimnological analysis represents one of the few data sources capable of revealing the timing and magnitude of biological changes that have occurred in stressed shallow lake environments on a decadal-centenial scale. This is an especially valuable technique at sites where previous monitoring of ecological and chemical condition has either never been performed, or which began after ecological degradation occurred. The determination

of the temporal variability of TBT contamination has shown that this compound leaves a clear profile subject to little diagenetic alterations, except a small amount of physical mixing. These contaminant characteristics enable definition of the temporal window in which ecotoxicological stress from the toxicant would have been operative within the lake.

The enumeration of biological remains from sediment cores can be seen as a record capable of revealing the longer-term influences of the multitude of internal and external factors shaping shallow lake ecosystems. By their nature, toxic pollution events are often short lived (less than a year), therefore identification of ecological processes causing ecosystem change from such relatively short time scale events, is beyond the capability of palaeoecological study in shallow lakes. However, the seasonal contamination of TBT in the waterways of the Broads occurred for over twenty years. The sensitivity of exposed taxa and the actual effects upon wider ecosystem structure within shallow lakes remains to be determined. However, the ecological decline observed at the onset of TBT contamination, and the subsequent failure to recover, as determined in the present study, is strongly suggestive that TBT has had some influence in shaping the Broads ecosystem.

CHAPTER 7 – SUMMARY AND CONCLUSIONS

7.1 Introduction

The overall aim of the present study was to reconstruct (through a spatio-temporal assessment of antifoul biocide concentrations in the River Bure waterway, in conjunction with a palaeolimnological approach) the changing ecology of two shallow lakes and determine whether TBT could have been a contributing factor in the switch from macrophyte to algal-dominated state. Analysis of water and surface sediments for contemporary antifoul biocide concentrations was carried out to improve understanding of the transport mechanisms that would have been responsible for dilution and dispersion of TBT during the period of its active usage. The presentation of results in the previous chapters has followed a logical progression which demonstrates how each objective was met. This chapter aims to briefly summarise the key findings of these chapters and give general conclusions and the wider implications of the study. Also included is a section on how research in the area could be further developed.

7.2 Summary

7.2.1 Organic biocide determination and quantification (Chapter 3)

Development of a novel method for the unequivical identification and subsequent low parts per trillion quantification of organic AFP biocides was a crucial first step in determining the spatial variability of boat-derived contamination. The analytical challenges of compound extraction, separation, ionisation, identification and quantification from complex environmental matrices were met using the online SPE-HPLC-APCI-MS^n methodology outlined in Chapter 3. Key successes were the good repeatability and reproducability of the method between sampling occasions; the low limits of detection achieved, especially for triazine biocides; fast, efficient extraction of triazines from sediment using a specifically developed MASE method; and effective reduction of errors in biocide quantification through identification of interferrence effects from compounds co-extracted from the sample matrix.

7.2.2 Spatial and temporal variation of organic biocide contamination in the River Bure waterway (Chapter 4)

The water sampling regime carried out between April 2003 and August 2004 successfully established the presence of a contamination gradient in organic AFP biocides within the River Bure and its associated boatyards and broads. Density of moored boats and proximity to areas of hull maintenance activities explained the presence of high concentration levels of Irgarol 1051 and diuron within boatyards. Lower contamination levels were detected in other navigable sites and in broads hydrologically connected to the main navigable river, but with no direct access for boating traffic. Surprisingly, the data between these two site types did not display a significant difference in AFP biocide concentrations, despite there being such a marked difference in direct exposure to boating activity. No broad isolated from navigation had any detectable AFP biocides present in water or sediments. Overall this spatial pattern confirmed that environmental contamination of these biocides was derived from that leached from AFP-coated boats. Temporal trends were also observed in Irgarol water concentrations that matched the seasonal variability in boating activity. At the river scale studied, the level of boating activity at each site had more of an influence over the variation in Irgarol surface sediment concentration than did the physical character of the sediments themselves.

7.2.3 Spatial and temporal variability of TBT contamination in the River Bure waterway (Chapter 5)

Quantifiable concentrations of TBT and its degradation products were determined from the surface sediments of the River Bure waterway dispite there being over 17 years since TBT was banned from use in AFPs on boats within the Broads. The variation in TBT surface sediment concentrations between sample sites exhibited a very similar spatial pattern and thus contamination gradient, as determined for the organic AFP biocides. A comparison with previously reported TBT surface sediment concentrations suggests that the maximum levels of TBT determined in the present study rank among the highest observed in freshwaters globally. The concentration ratio of TBT to its primary breakdown product (TBT/DBT) within the most contaminated hotspots, indicated a very low rate of degradation, with TBT concentrations outside of boatyards not significantly lower than those reported from

the same area over 15 years beforehand. It is thought that redistribution from highly contaminated areas has occurred.
The transportation of aqueous and sediment-bound AFP biocides was demonstrated by the intensive sampling of a single connected broad. Thorough mixing of the relatively soluble triazine biocides was found across Ranworth Broad, which helps to explain the lack of difference in aqueous concentrations between navigable and hydrologically connected sites. This spatial pattern also validated the use of "spot" samples to characterise the aqueous contamination within each broad. Variation in the concentration of TBT in surface sediments of Ranworth Broad was related to distance from the inlet, suggesting horizontal transport of contaminated sediments. No significant relationship between the sediment characteristics measured and AFP biocide concentration were found, suggesting that, at the river scale, the location of a sample site in relation to the pattern of boating activity was of greater influence.

Temporal variation of TBT persisting within the stratigraphical record of several broads was successfully determined from radimetrically dated sediment cores. Similar TBT contamination profiles were obtained from the navigable Salhouse Great and Wroxham Broad (latter reported in Sayer *et al* (2006). There was also variation in the timing of initial detection of TBT signatures in the Bure broads, which were slightly earlier (early-mid 1960's) than in Hickling Broad (late 1960's). The radiometric dating of Bure broads cores proved to be of limited resolution, but the dates of TBT first appearing in these sediment profiles was predicted to be in the mid 1960s. Initial detection of TBT, in the more reliably dated core from Hickling Broad, suggested that first appearance occurred in the late 1960s at this site. Core results from the connected Hoveton Great Broad demonstrated that even broads not directly exposed to boating activity were contaminated by TBT during the period of its active usage.

7.2.4 Palaeoecological change at the onset of TBT contamination (Chapter 6)

Use of a novel wide-diameter Piston corer facilitated the collection of cores suitable for detailed multi-proxy palaeolimnological analysis from recent shallow lake sediments. All cores analysed in this way displayed a progressive loss of macrophytes and associated fauna. Salhouse Broad appeared to have already lost some if its submerged flora long before the introduction of TBT, similar to Wroxham Broad (Sayer et al. 2006). Various biological changes have been shown to occur in

synchrony with the first detection of TBT, but in Hoveton Great and Hickling Broads, where macrophyte dominance persisted much later than in Salhouse Broad, the magnitude of change coincident with TBT usage was far greater. The extent to which TBT precipitated such changes remains unclear, as further palaeolimnological study of control sites and determination of the toxicants bioavailability to organisms is required. The present study has demonstrated that multiproxy, including macrofossil analysis, is a valuable tool with which to determine historic shifts in macrophyte to planktonic dominance within shallow lakes.

7.3 Management issues and future directions

Knowledge of the stressors acting to degrade shallow lakes, or prevent their subsequent recovery, are clearly sought if effective ecosytsem restoration is to be achieved. Macrophyte recovery in the Bure broads most contaminated by AFP biocides has not been apparent, even after the ban on TBT usage (Broads Authority 2004). Recent work has also demonstrated the role that increased salinity plays in reducing the stability of the macrophyte dominated state within coastal shallow lakes (Barker *et al.* 2007), so separating mulitple stressors acting to degrade such ecosystems is key to effective management. Also, the relatively high TBT concentration levels in contemporary surface sediments represents an on-going pollution problem. There is still a risk of TBT entering the aquatic food web through biotic uptake from contaminated surface sediments.

Activities such as maintenance dredging which disturb sediment, including that buried at anoxic depths below the sediment surface, will act to redistribute and promote desorbtion of TBT to the overlying waters. Identification of the potential risk posed by TBT and other boat-derived contaminants, prior to such works, is clearly desirable for sustainable environmental management. At locations where such risk is predicted to be high, suitable mitigation measures may assist in the reduction of further negative biological effects. However, further study into the quantifiable risk posed by TBT released from sediments is required to define the magnitude of the current problem. The risk posed by such sediment-bound contamination and its secondary pollution effects is relatively little studied in freshwater systems.

Future study could aim to determine the interaction between the sediment-bound TBT and its equilibrium with the pore water and the potential for TBT release to the

overlying water column. Modelling of the TBT sediment-water dynamics within contaminated waterways would help establish the ecotoxicological risk posed to aquatic biota from secondary release. This research could take the form of a combined approach in quantifying TBT desorbtion from contaminated sediments and laboratory based toxicological tests. Uncertainty in the cause and effect relationship between TBT exposure and biotic response in the natural environment could be further reduced by performing in-situ ecotoxicological testing at known contaminated sites. This would allow intercalibration of the modelled biological resposes derived from standard toxicity tests and those observed under field conditions.

A study modelling TBT sorption and subsequent degradation in cores would also provide quantitative limits for the dissolved concentrations likely to have occurred at the time of deposition. This would assist in determination of the causality of biological changes, as inferred from palaeoecological studies, through better understanding of the environmental pathway of TBT from source to sink.

A global ban on TBT use in antifoulant coatings was implemented in 2003 by the International Maritime Organisation (van Wezel and van Vlaardingen 2004b). An increased risk of market forces deferring TBT usage to non-signatory, mainly developing nations, with weak environmental monitoring and regulatory frameworks has been discussed (Champ 2000). If this is so, then TBT usage may already, or more frequently have deleterious impacts within navigable lakes where managers and stakeholders are less able to respond to the problem of TBT contamination. Further development and validation of research methodologies capable of separating environmental stressors acting upon aquatic ecosystems are therefore highly valuable if further environmental degradation is to be minimised.

7.4 Conclusions

AFP biocide spatial distributions were successfully determined in the River Bure waterway. Concentrations of modern organic AFP biocides and the persistent TBT were found to be present found in an environmental contamination gradient that related to the level and type of boating activity. It is clear that TBT was a widespread contaminant within River Bure waterway, with significant concentrations penetrating into connected waterbodies not directly open to navigation. The dating of sediment depths when TBT was first detected suggest that there was a continuous period of

TBT exposure that lasted from at least the late 1960s until several years after the compound was banned in 1987. Previous work suggests that TBT concentrations present in a bioavailable, dissolved form, in the late 1980s and early 1990s, was sufficient to have caused numerous acute and chronic responses in freshwater fauna. The environmental persistence of TBT is such that considerable concentrations are still extant in contemporary surface sediments and continues to pose a risk to aquatic biota. Analysis of macrofossil and cladoceran remains has shown that in TBT exposed lakes which had maintained macrophyte growth in the face of increased nutrient levels, a synchronous loss of the macrophyte dominated state occurred. The extent to which these changes and the exact role that TBT played in this shift is not clear, but the synchronicity of the timings means it cannot be ruled out as a causal factor.

The present study has shown that the Broads aquatic environment is currently and has historically been an area of intense antifoulant biocide contamination when compared to other recreational boating locations. The combination of spatial and temporal approaches in determining variation of antifoulant biocide exposure provides a powerful space-time framework with which to analyse the environmental fate of such contaminants. Given the potential toxicity of TBT towards aquatic biota, it is perhaps surprising that this compound has not received more attention in studies of ecosystem degradation in navigable freshwaters, as has been the case in the marine environment. However within the Broads, the density of craft compared to total water volume and the limited water exchange have created conditions that have increased the potential for widespread AFP biocide contamination. Generally, previous studies of AFP contamination in freshwaters have been in larger continental lakes and major river systems where dilution effects are greater (Fent and Hunn 1995;Loganathan *et al.* 2001). The navigable waterways of the Broads therefore represent an ideal area for studying the role of TBT exposure in the degradation shallow lakes. An interaction between this toxic stressor and an already nutrient enriched ecosystem has been highlighted, producing variability in the ecological response to such pollution between different sites. The spatial and temporal sampling framework for determining toxicant variability at the catchment scale provides a powerful tool with which to assess the relative intensity and duration of toxicant exposure within such complex wetland systems.

In terms of a toxicant acting as a forward switch in the catastrophic loss of macrophytes from shallow lakes, TBT can be considered as a strong candidate. The numerous direct and indirect ecotoxicological responses to TBT exposure, displayed by key functional organism groups, therefore represents the kind of perturbation required by current understanding of the alternative equilibrium theory. In the present study, evidence from Hoveton Great and Hickling Broad is interpreted as suggesting that an indirect promotion of algal dominance was caused by the TBT induced breakdown of internal ecological buffering mechanisms, which prior to contamination, maintained conditions conducive for macrophyte growth. In the heavily contaminated Salhouse Broad, further degradation of the already depleted, eutrophic lake condition was observed. As increases in the nutrient content of Broads water were already underway prior to the introduction of TBT, the potential for algal dominance was enhanced, with a final shift from benthic to pelagic production occurring in synchrony with the onset of TBT pollution. The decreased ecological resilience present in this shallow lake ecosystem has meant that the potential for recovery from a perturbation, such as prolonged TBT contamination, has been much reduced.

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9.0 APPENDICES

9.1 Biocide physio-chemical properties

Table 9.1 Irgarol 1051

2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine	$C_{11}H_{19}N_5S$
CAS no. 28159-98-0	Molecular mass: 253.4

Property	Value	Reference
Water solubility (mg l^{-1})		
(0 NaCl mol l^{-1})	9	
(0.3)	5.9	
(0.6 seawater)	1.8	(Ciba Chemicals Inc. 1999)
log K_{ow}	3.4	(Lam *et al.* 2006)
	2.8	(Ciba Chemicals Inc. 1999)
	4.0	(Bard and Pedersen 1992)
	4.1	SRC (1997) [a]
log K_{oc}	2.38	(Hall *et al.* 1999)
	3.0	(Tolosa *et al.* 1996)
	2.4 - 4.9	(Comber *et al.* 2001)
	>3.56	(Ciba Chemicals Inc. 1998)
	4.3 - 6.3	(Bowman *et al.* 2003)
Vapour pressure (m Pa)		
@25 °C	0.088	(Ciba Chemicals Inc. 1999)
	0.015	(Hall *et al.* 1999)

[a] cited in (van Wezel and van Vlaardingen 2004b)

Table 9.2 Diuron

3-(3,4-dichlorophenyl)-1,1-dimethylurea		$C_9H_{10}Cl_2N_2O$
CAS no. 330-54-1		Molecular mass: 233.1
Property	**Value**	**Reference**
Water solubility mg l^{-1}	42	Worthing & Hance (1991) [a]
log K_{ow}	2.85	Howard (1990) [a]
log K_{oc}	2.6	Rao *et al* (1982) [a]
	2.82	Peck *et al* (1980) [b]
Vapour pressure (m Pa)		
@20 °C	0.002	(HSE 2001)

[a] cited in (Lewis and Gardiner 1996)

[b] cited in (Comber *et al.* 2001)

Table 9.3 Atrazine

(1-methylethyl)-1,3,5-triazine-24-diamine		$C_8H_{14}ClN_5$
CAS no. 1912-24-9		Molecular mass: 215.7
Property	**Value**	**Reference**
Water solubility mg l^{-1}	33	Tyson (1985) [a]
log K_{ow}	2.7	Hedgecott & Zabel (1991) [a]
	2.6	(Paschke *et al.* 2004)
log K_{oc}	2.23	Hedgecott & Zabel (1991) [a]
	2.1	Squillace & Thurman (1992) [b]
	2.36	(USEPA 2004)
Vapour pressure (m Pa)		
@20 °C	0.04	Hedgecott & Zabel (1991) [a]

[a] cited in (HSE 1993)

[b] cited in (Tolosa *et al.* 1996)

Table 9.4 Isoproturon

3-(4-isopropylphenyl)-1,1-dimethylurea	$C_{12}H_{18}N_2O$
CAS no. 1912-24-9	Molecular mass: 206.3

Property	Value	Reference
Water solubility mg l^{-1}	55	Worthing & Hance (1991) [a]
log K_{ow}	2.2	Worthing & Hance (1991) [a]
log K_{oc}	2.7	Moore & Gibby (1990) [a]
	2.24	(Nemeth-Konda *et al.* 2002)
Vapour pressure (m Pa)		
@20 °C	0.0033	Worthing & Hance (1991) [a]

[a] cited in (Lewis and Gardiner 1996)

Table 9.5 Tributyltin
(representative data for bis(tributyltin)oxide given)

CAS no. 56-35-9	$C_{24}H_{54}OSn_2$
Molecular mass: 596	

Property	Value	Reference
Water solubility mg l^{-1}	31	(Maguire *et al.* 1983)
	19.5	(Zabel *et al.* 1988)
log K_{ow}	4.1	(Arnold *et al.* 1997)
	4.42	(Becker van Slooten and Tarradelas
1994) 3.74		de Mora (1996) [a]
log K_{oc}	4.5	(Meador 2000)
	4.15	(Becker van Slooten and Tarradelas 1994)

[a] cited in (Voulvoulis *et al.* 2002)

9.2 Organic biocide water concentrations

Table 9.6 Seasonal water concentrations (ng l^{-1}) of Irgarol and diuron

Irgarol	Apr-03	Aug-03	Nov-03	Feb-04	May-04	Aug-04
Isolated m/z (product ion)	254 (198)	254 (198)	254 (198)	254 (198)	254 (198)	254 (198)
Calibration range ng l^{-1}	20 - 1000	2 - 1000	2 - 1000	2 - 1000	2 - 1000	2 - 1000
Correlation coefficient r^2	0.99924	0.99020	0.99959	0.99971	0.99384	0.99983
RSD	3.3	9.1	6.2	6.4	8.9	1.5
u/s Horstead Mill	-	-	-	-	-	-
u/s Wroxham Rail Bridge	<20	-	-	-	-	<2
u/s Wroxham Broad	-	**9**	**8**	-	<2	**5**
u/s Decoy Broad	<20	**4**	**8**	-	**2**	**6**
u/s Ranworth Broad	**29**	**8**	**9**	-	**4**	**7**
Wroxham Broad	<20	**7**	**8**	-	**4**	**8**
Salhouse Great Broad	-	**4**	**7**	-	**3**	**6**
Hoveton Great Broad	<20	**3**	-	-	**3**	**4**
Cockshoot Broad	-	-	-	-	-	-
Ranworth Broad	**22**	**4**	**9**	-	**3**	**5**
Connoisseur	**32**	**15**	**10**	**16**	**20**	**20**
Loynes	<20	**3**	**9**	**2**	**3**	**5**
Barnes Brinkcraft	<20	**3**	**8**	-	<2	**3**
Landamores	**57**	**21**	**12**	**8**	**10**	**24**
Horning Ferry Marina	> 1000	**258**	**34**	**22**	**108**	**195**

Diuron	Apr-03	Aug-03	Nov-03	Feb-04	May-04	Aug-04
Isolated m/z (product ion)	nd	233	233	233	233	233
Calibration range ng l^{-1}		50 - 1000	50 - 1000	20 - 1000	20 - 1000	20 - 1000
Correlation coefficient r^2		0.99554	0.99887	0.99981	0.99419	0.99530
RSD		1.2	4.6	7.5	15.5	9.8
u/s Horstead Mill		-	-	-	-	-
u/s Wroxham Rail Bridge		-	-	-	-	-
u/s Wroxham Broad		-	-	-	-	-
u/s Decoy Broad		-	-	-	-	-
u/s Ranworth Broad		<50	-	-	-	-
Wroxham Broad		<50	-	-	-	-
Salhouse Great Broad		<50	-	-	-	-
Hoveton Great Broad		-	-	-	-	-
Cockshoot Broad		-	-	-	-	-
Ranworth Broad		-	-	-	-	-
Connoisseur		<50	-	-	**51**	<20
Loynes		-	<50	-	<20	-
Barnes Brinkcraft		-	-	-	<20	-
Landamores		**133**	<50	<20	**45**	-
Horning Ferry Marina		**550**	<50	<20	**154**	**223**

- no peak detected
29 quantified concentration
nd not analysed
<20 S/N acceptable, but < LOQ

Table 9.7 Seasonal water concentrations (ng l^{-1}) of atrazine and isoproturon

Atrazine	Apr-03	Aug-03	Nov-03	Feb-04	May-04	Aug-04
Isolated m/z (product ion)	nd	216 (174)	216 (174)	216 (174)	216 (174)	216 (174)
Calibration range ng l^{-1}		10 - 1000	10 - 1000	10 - 1000	10 - 1000	10 - 1000
Correlation coefficient r^2		0.99711	0.99937	0.99878	0.99643	0.99772
RSD		2.2	0.7	2.2	9.5	5.6
u/s Horstead Mill		**17**	**16**	<10	**28**	**24**
u/s Wroxham Rail Bridge		**38**	**19**	<10	**30**	**23**
u/s Wroxham Broad		**44**	**20**	<10	**28**	**24**
u/s Decoy Broad		**23**	**25**	<10	**33**	**28**
u/s Ranworth Broad		**25**	**23**	<10	**25**	**43**
Wroxham Broad		**40**	**27**	<10	**34**	**24**
Salhouse Great Broad		**20**	**26**	**13**	**35**	**24**
Hoveton Great Broad		**22**	**23**	**14**	**30**	**23**
Cockshoot Broad		<10	<10	-	<10	**10**
Ranworth Broad		**25**	**18**	**12**	**17**	**34**
Connoisseur		**45**	**26**	**13**	**29**	**20**
Loynes		**42**	**32**	**13**	**47**	**24**
Barnes Brinkcraft		**40**	**23**	**13**	**43**	**21**
Landamores		**44**	**32**	**22**	**45**	**29**
Horning Ferry Marina		**28**	**20**	**15**	**17**	**30**

Isoproturon	Apr-03	Aug-03	Nov-03	Feb-04	May-04	Aug-04
Isolated m/z (product ion)	nd	207 (165)	nd	207 (165)	207 (165)	207 (165)
Calibration range ng l^{-1}		50 - 1000		20 - 1000	10 - 1000	10 - 1000
Correlation coefficient r^2		0.99559		0.99733	0.99296	0.99681
RSD		3.94		12.6	8.7	2.6
u/s Horstead Mill		-		<20	**17**	-
u/s Wroxham Rail Bridge		-		<20	**21**	-
u/s Wroxham Broad		-		<20	**19**	-
u/s Decoy Broad		-		**47**	**34**	-
u/s Ranworth Broad		-		**57**	**19**	-
Wroxham Broad		-		<20	**29**	-
Salhouse Great Broad		-		**48**	**42**	-
Hoveton Great Broad		-		**52**	**41**	-
Cockshoot Broad		-		-	-	-
Ranworth Broad		-		-	**17**	-
Connoisseur		-		<20	**22**	-
Loynes		-		<20	**32**	-
Barnes Brinkcraft		-		**92**	**51**	-
Landamores		-		**39**	**29**	-
Horning Ferry Marina		-		**47**	**14**	-

- no peak detected
17 quantified concentration
nd not analysed
<20 S/N acceptable, but < LOQ

Table 9.8 Organic biocide water concentrations (ng l^{-1}) August 2004

		Irgarol	Diuron	Atrazine	Isoproturon
	Isolated m/z (product ion)	254 (198)	233	216 (174)	207 (165)
	Calibration range ng l^{-1}	2 - 1000	20 - 1000	10 - 1000	10 - 1000
	Correlation coefficient r^2	0.99983	0.99530	0.99772	0.99681
	RSD	1.5	9.8	5.6	2.6
d/s WWTW					
	d/s Briston works	-	-	**17**	-
	d/s Aylsham works	-	-	**17**	-
	d/s Coltishall works	-	-	**39**	-
	d/s Rackheath works	-	-	**21**	-
	d/s Belaugh works	-	-	**24**	-
river mid-channel					
	u/s Horstead Mill	-	-	**24**	-
	u/s Wroxham Rail Bridge	<2	-	**23**	-
	u/s Wroxham Broad	**5**	-	**24**	-
	u/s Decoy Broad	**6**	-	**28**	-
	u/s Ranworth Broad	**7**	-	**43**	-
	River Ant	**6**	-	**67**	<10
	u/s South Walsham Broad	**7**	-	**43**	-
Isolated broads					
	Burntfen Broad	-	-	<10	-
	Woodbastwick Turfpond	-	-	**11**	-
	Cockshoot Broad	-	-	**10**	-
	Pedham Lake	-	-	**12**	-
	Upton Broad	-	-	**11**	-
Connected broads					
	Hudson's Bay	**3**	-	**24**	-
	Salhouse Little Broad	**6**	-	**21**	-
	Hoveton Great Broad	**4**	-	**23**	-
	Decoy Broad	**4**	-	**39**	-
	Ranworth Broad	**5**	-	**34**	-
Navigable broads					
	Wroxham Broad	**8**	-	**24**	-
	Salhouse Great Broad	**6**	-	**24**	-
	Hoveton Little Broad	**4**	-	**33**	-
	Malthouse Broad	**10**	**36**	**29**	-
	South Walsham Broad	**7**	-	**11**	-
Boatyards/marinas					
	Connoisseur	**20**	<20	**20**	-
	Loynes	**5**	-	**24**	-
	Barnes Brinkcraft	**3**	-	**21**	-
	Landamores	**24**	-	**29**	-
	Horning Ferry Marina	**195**	**223**	**30**	-

- no peak detected
4 quantified concentration
nd not analysed
<20 S/N acceptable, but < LOQ

9.3 Radionuclide stratigraphy

(analysis by P.G. Appleby, ERRC, University of Liverpool)

9.3.1 Salhouse Broad (SALG1)

Lead-210 Activity

Total ^{210}Pb activity was significantly in excess of that of the supporting ^{226}Ra only in the uppermost sample (Figure 9.1a). In all deeper samples, unsupported ^{210}Pb activity (Figure 9.1b) was either close to or below the limit of detection.

Artificial Fallout Radionuclides

The ^{137}Cs activity versus depth profile (Figure 9.1c) is a little irregular, though it does have a relatively well defined peak between 32.5-40.5 cm that almost certainly records the 1963 fallout maximum from the atmospheric testing of nuclear weapons.

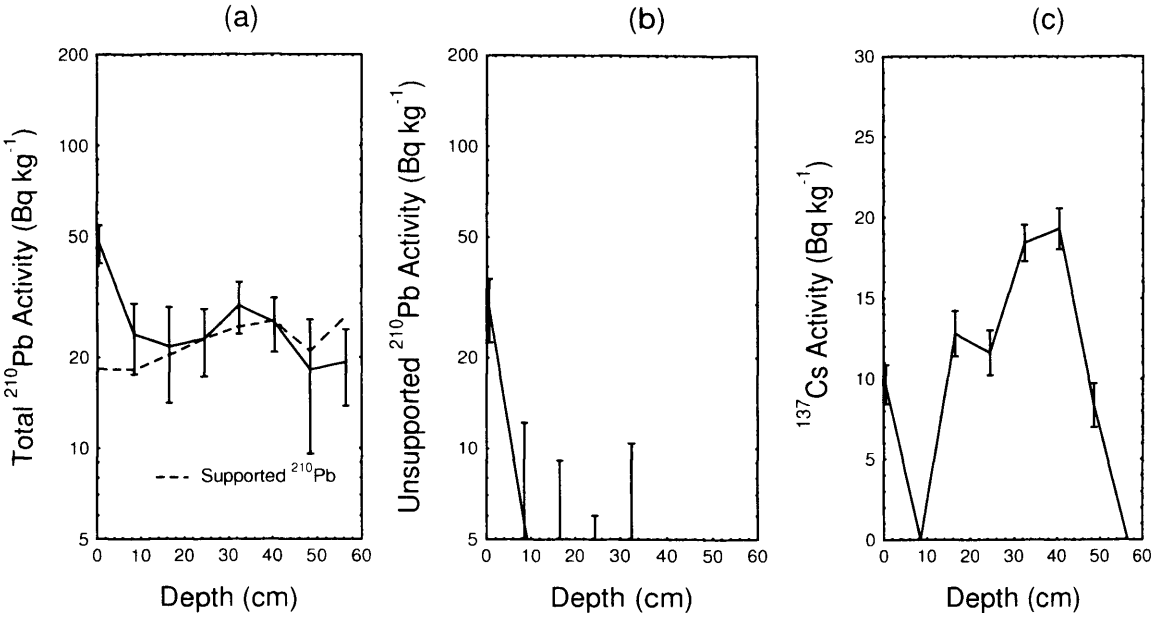

Figure 9.1 Fallout radionuclides in the Salhouse Broad core SALG1 showing (a) total and supported ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs concentrations versus depth.

Because of the poor ^{210}Pb record it was not possible to date this core by ^{210}Pb. The ^{137}Cs record does however show that the top 40 cm of the core only spans around 40 years. From the ^{137}Cs date, the mean sedimentation rate during this period is calculated to be 0.28 ± 0.03 g cm^{-2} y^{-1} (0.91 cm y^{-1}).

Table 9.9 Radionuclide concentrations in Salhouse Broad core SALG1

Depth		^{210}Pb Total		^{210}Pb Unsupported		^{210}Pb Supported		^{137}Cs	
cm	g cm^{-2}	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±
0.5	0.1	47.6	6.8	29.4	7.0	18.3	1.7	9.6	1.2
8.5	1.8	23.8	6.3	5.6	6.5	18.2	1.8	0.0	0.0
16.5	4.2	21.6	7.5	1.4	7.7	20.2	1.8	12.8	1.4
24.5	7.0	23.0	5.8	0.1	6.0	22.9	1.5	11.6	1.4
32.5	9.7	29.6	5.8	4.4	6.0	25.1	1.6	18.4	1.1
40.5	12.3	26.0	5.3	-0.3	5.5	26.3	1.7	19.3	1.3
48.5	14.7	18.1	8.5	-2.8	8.7	20.9	1.9	8.4	1.4
56.5	17.4	19.2	5.4	-8.0	5.6	27.2	1.4	0.0	0.0

Table 9.10 gives a radiometric chronology calculated on this basis. From these results, shown also in Figure 9.2, it does appear that the poor ^{210}Pb record is simply due to dilution of the atmospheric flux by very rapid sedimentation. The surficial unsupported ^{210}Pb concentration of 29 Bq kg^{-1} corresponds to ^{210}Pb flux of ~80 Bq m^{-2} y^{-1}, a figure that is close to the estimated atmospheric flux.

1.1

Table 9.10 Radiometric chronology of Salhouse Broad core SALG1

Depth		Chronology Date	Age		Sedimentation Rate		
cm	g cm^{-2}	AD	y	±	g cm^{-2} y^{-1}	cm y^{-1}	± (%)
0.0	0.00	2003					
0.5	0.1	2003	0	0	0.28	1.48	12.3
4.5	0.8	2000	3	0	0.28	1.26	12.3
8.5	1.8	1996	7	1	0.28	1.02	12.3
12.5	3.0	1992	11	1	0.28	0.93	12.3
16.5	4.2	1988	15	2	0.28	0.87	12.3
20.5	5.5	1983	20	2	0.28	0.79	12.3
24.5	7.0	1978	25	3	0.28	0.77	12.3
28.5	8.4	1973	30	4	0.28	0.80	12.3
32.5	9.7	1968	35	4	0.28	0.83	12.3
36.5	11.0	1963	40	5	0.28	0.86	12.3
40.5	12.3	1958	45	5	0.28	0.90	12.3
44.5	13.5	1954	49	6	0.28	0.91	12.3
48.5	14.7	1950	53	7	0.28	0.87	12.3
52.5	16.0	1945	58	7	0.28	0.82	12.3
56.5	17.4	1940	63	8	0.28	0.80	12.3

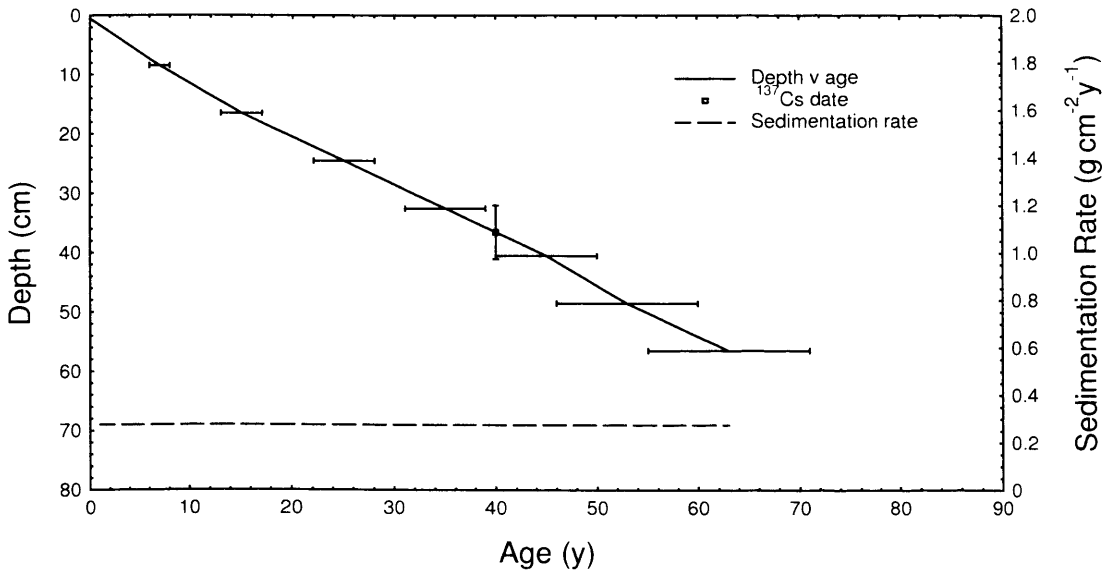

Figure 9.2 Radiometric chronology of Salhouse Broad core SALG1 (showing the 1963 depth determined from the ^{137}Cs stratigraphy and the sediment depths versus age assuming a constant dry mass sedimentation rate)

9.3.2 Hoveton Great Broad (HGBO1)

Lead-210 Activity

Total ^{210}Pb activity significantly exceeding that of the supporting ^{226}Ra was detected only in the top 20 cm of the core (Figure 9.3a). Unsupported ^{210}Pb activities in this section of the core were extremely low (Figure 9.3b), with a maximum value of just 21 Bq kg^{-1}. The ^{210}Pb dating horizon for this core is thus likely to be no more than around two half-lives (between 40-50 years). The ^{210}Pb inventory of the core (398 Bq m^{-2}) corresponds to a mean ^{210}Pb flux of just 12 Bq m^{-2} y^{-1}, significantly lower than the mean value recorded in other Norfolk Broads of 24 Bq m^{-2} y^{-1} (Appleby, unpublished data).

Artificial Fallout Radionuclides

^{137}Cs activity versus depth record (Figure 9.3c) has a relatively well-resolved peak at 16.5 cm that may record the 1963 fallout maximum from the atmospheric testing of nuclear weapons. In view of the extremely low concentrations (maximum value 8 ± 1 Bq kg^{-1}) the reliability of this feature is open to question.

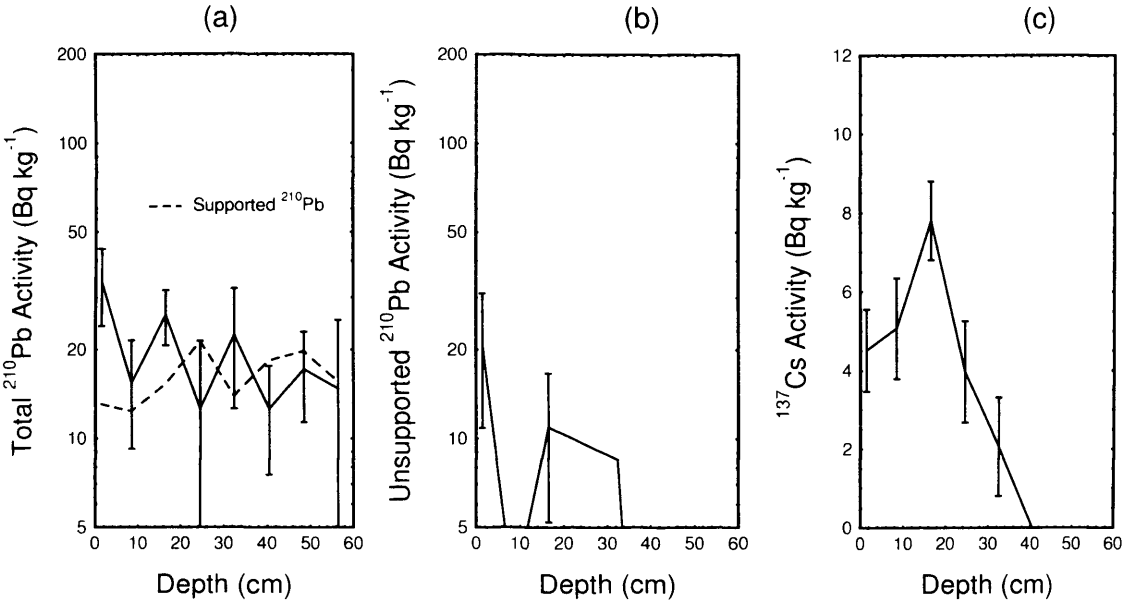

Figure 9.3 Fallout radionuclides in the Hoveton Great Broad core HGB01 showing (a) total and supported ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs concentrations versus depth.

Table 9.11 Radionuclide concentrations in Hoveton Great Broad core HGBO1 1.2

Depth		^{210}Pb Total		^{210}Pb Unsupported		^{210}Pb Supported		^{137}Cs	
cm	g cm^{-2}	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±
1.5	0.2	34.0	10.0	20.9	10.0	13.0	1.4	4.5	1.0
8.5	1.5	15.3	6.1	3.0	6.3	12.4	1.4	5.1	1.3
16.5	3.5	26.2	5.5	10.9	5.7	15.3	1.4	7.8	1.0
24.5	5.7	12.6	8.7	-8.7	8.9	21.3	1.9	4.0	1.3
32.5	7.6	22.4	9.8	8.5	10.0	13.9	1.8	2.1	1.3
40.5	9.6	12.6	5.0	-5.8	5.3	18.4	1.5	0.0	0.0
48.5	12.0	17.1	5.8	-2.6	6.1	19.7	1.6	0.0	0.0
56.5	14.3	14.7	10.4	-0.8	10.6	15.6	1.9	0.0	0.0

1.3

Table 9.12 ^{210}Pb chronology of Hoveton Great Broad core HGB01

Depth		Chronology			Sedimentation Rate		
cm	g cm^{-2}	Date AD	Age y	±	g cm^{-2} y^{-1}	cm y^{-1}	± (%)
0.0	0.00	2005	0	0			
4.5	0.72	1997	8	2	0.085	0.47	13.5
8.5	1.55	1987	18	3	0.085	0.39	13.5
12.5	2.48	1976	29	5	0.085	0.34	13.5
16.5	3.54	1963	42	7	0.085	0.31	13.5
20.5	4.67	1950	55	8	0.085	0.32	13.5

Figure 9.4 shows a best estimate of the 1963 depth determined from the ^{137}Cs stratigraphy, together with ^{210}Pb dates calculated using the ^{137}Cs date as a reference point (Appleby 2002). Since the ^{210}Pb record was evidently incomplete, it could not be used on its own to calculate a sediment chronology. Various estimates of the sedimentation rate based on the available data suggest a mean value of 0.085 ± 0.015 g cm^{-2}y^{-1} (0.39 cm y^{-1}). Table 9.12 gives sediment dates calculated using this value.

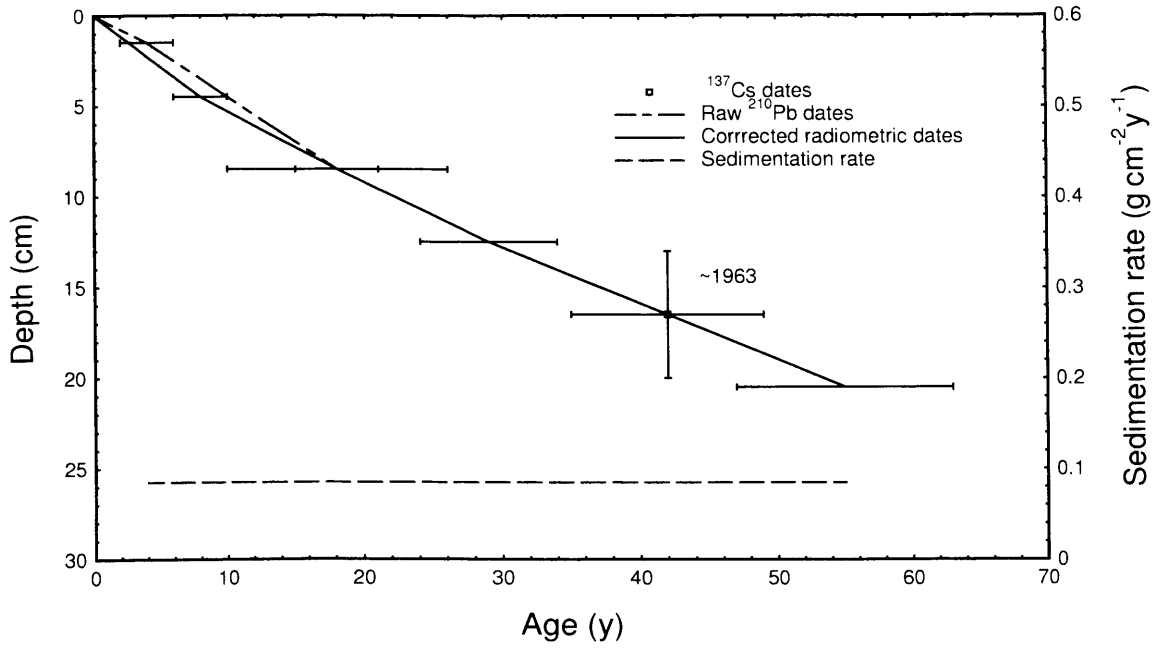

Figure 9.4 Radiometric chronology of Hoveton Great Broad core HGB01 (showing the raw ^{210}Pb dates, the approximate 1963 depth determined from the ^{137}Cs record, and the corrected radiometric dates and sedimentation rates)

9.3.3 Hickling Broad (HICK1)

Lead-210 Activity

Total ^{210}Pb activity reaches equilibrium with the supporting ^{226}Ra at a depth of around 23 cm (Figure 9.5a). Unsupported ^{210}Pb activities, calculated by subtracting ^{226}Ra activity from total ^{210}Pb activity, declines relatively slowly with depth in the top 10 cm of the core. At this point there is a relative abrupt change in the gradient of the profile (Figure 9.5b), with a much steeper rate of decline in the deeper sediments. Within this deeper zone the profile more or less follows an exponential relationship, apart from a possible non-monotonic feature near the base of the ^{210}Pb record.

Artificial Fallout Radionuclides

^{137}Cs activity versus depth profile (Figure 9.5c) has relatively well resolved peak between 13-16 cm that almost certainly records the 1963 fallout maximum from the atmospheric testing of nuclear weapons. This interpretation is supported by the detection of traces of ^{241}Am in the 14.5-15.5 cm sample.

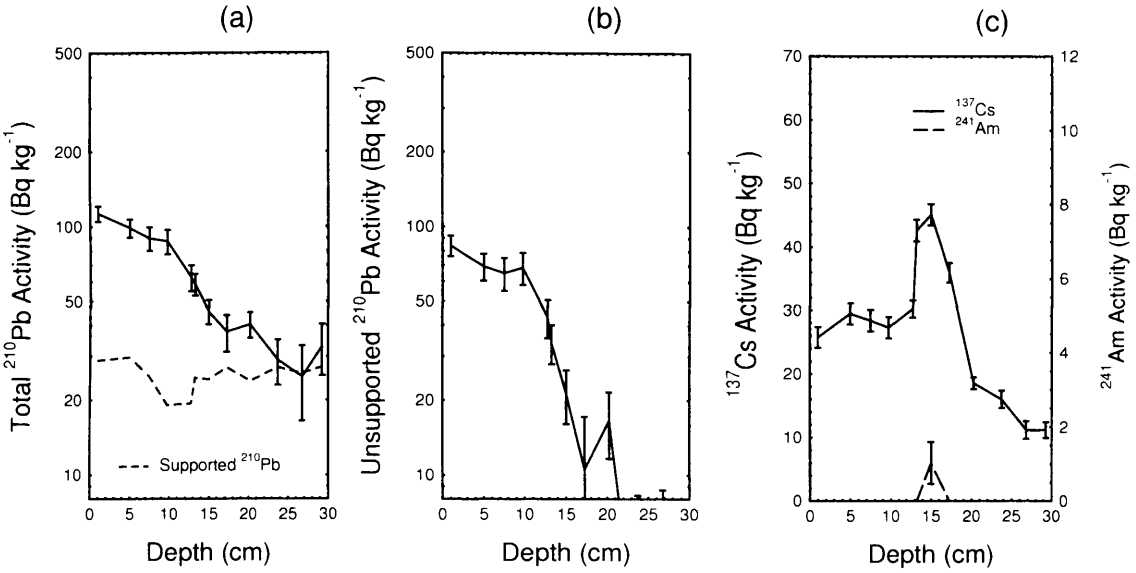

Figure 9.5 Fallout radionuclides in the Hickling Broad core HICK1 showing (a) total and supported ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs and ^{241}Am concentrations versus depth.

Figure 9.6 shows ^{210}Pb dates calculated using the CRS and CIC dating models (Appleby and Oldfield 1978), together with the 1963 depth determined from the ^{137}Cs stratigraphy. The CRS model places 1963 at a depth of 11.75 cm, significantly above the depth suggested by the ^{137}Cs record. The CIC model gives a better agreement, placing 1963 at a depth of 14 cm, though dates calculated by this model

Table 9.13 Radionuclide concentrations in Hickling Broad core HICK1

Depth		^{210}Pb Total		^{210}Pb Unsupported		^{210}Pb Supported		^{137}Cs		^{241}Am	
cm	g cm^{-2}	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±
1.00	0.07	112.8	7.9	83.9	8.1	28.9	1.9	25.7	1.6	0.0	0.0
5.00	0.50	98.9	8.3	69.1	8.5	29.8	1.9	29.5	1.7	0.0	0.0
7.50	0.80	89.9	9.8	65.0	9.9	24.9	1.7	28.4	1.7	0.0	0.0
9.75	1.08	87.4	9.9	68.3	10.1	19.1	1.6	27.3	1.7	0.0	0.0
12.75	1.48	62.6	7.6	43.2	7.7	19.4	1.3	30.3	1.4	0.0	0.0
13.25	1.56	58.7	5.9	34.1	6.1	24.6	1.5	42.6	1.7	0.0	0.0
15.00	1.87	45.5	5.1	21.3	5.2	24.2	1.3	45.1	1.7	1.0	0.6
17.25	2.29	37.6	6.3	10.5	6.6	27.1	2.0	36.0	1.6	0.0	0.0
20.25	2.93	40.4	4.8	16.6	5.0	23.8	1.2	18.6	1.0	0.0	0.0
23.75	3.74	29.0	6.1	2.0	6.3	27.1	1.6	16.0	1.4	0.0	0.0
26.75	4.44	24.8	8.4	-0.9	8.6	25.7	2.0	11.2	1.4	0.0	0.0
29.25	5.01	32.8	7.7	5.6	7.9	27.2	1.8	11.2	1.3	0.0	0.0

are generally more irregular. Better results, also shown in Figure 9.6 and given in detail in Table 9.14, are obtained by the CRS model using the 1963 ^{137}Cs date as a reference point (Appleby 2002). These calculations suggestion that since 1950 sedimentation rates have fluctuated between 0.031-0.055 g cm^{-2} y^{-1}, with a mean value of 0.041 ± 0.009 g cm^{-2} y^{-1} (0.31 cm y^{-1}). They also suggest that sedimentation rates were significantly lower during the period 1920-50, though because of the low ^{210}Pb concentrations below 15 cm values from this period have a large uncertainty.

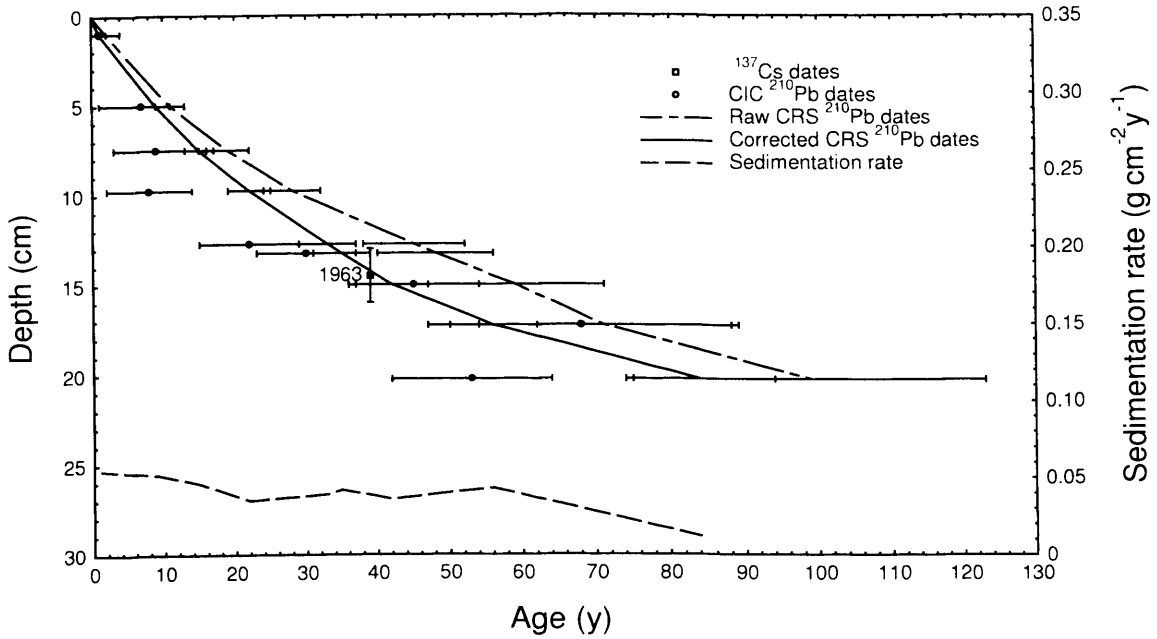

Figure 9.6 Radiometric chronology of Hickling Broad core HICK1 (Showing the CRS and CIC model ^{210}Pb dates and the 1963 depth determined from the ^{137}Cs stratigraphy. Also shown are the corrected ^{210}Pb dates and sedimentation rates calculated using the ^{137}Cs date as a reference level)

Table 9.14 ^{210}Pb chronology of Hickling Broad core HICK1

Depth		Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	y	±	g cm^{-2} y^{-1}	cm y^{-1}	± (%)
0.0	0.00	2002	0				
1.0	0.07	2001	1	1	0.055	0.67	11.9
2.0	0.18	1999	3	2	0.054	0.57	12.7
3.0	0.29	1997	5	2	0.053	0.50	13.4
4.0	0.40	1995	7	2	0.053	0.44	14.2
5.0	0.50	1993	9	2	0.052	0.44	14.9
6.0	0.62	1990	12	2	0.050	0.40	16.2
7.0	0.74	1988	14	2	0.047	0.36	17.4
8.0	0.86	1985	17	2	0.043	0.36	18.1
9.0	0.99	1982	20	2	0.039	0.31	18.3
10.0	1.11	1979	23	3	0.035	0.29	18.9
11.0	1.25	1975	27	3	0.037	0.29	20.5
12.0	1.38	1971	31	4	0.038	0.27	22.1
13.0	1.52	1968	34	4	0.043	0.26	23.6
14.0	1.69	1964	38	5	0.046	0.27	26.8
15.0	1.87	1960	42	9	0.037	0.21	30.8
16.0	2.06	1956	46	10	0.031	0.16	46.8
17.0	2.24	1949	54	11	0.042	0.14	62.7
18.0	2.45	1940	63	13	0.035	0.12	60.9
19.0	2.66	1931	72	15	0.025	0.11	53.3
20.0	2.88	1922	81	16	0.014	0.11	45.6

9.3.4 Barton Broad radiometric dating results

Lead-210 Activity

^{210}Pb concentrations in excess of the supporting ^{226}Ra were above limits of detection down to a depth of between 25-35 cm (Figure 9.7a). Unsupported ^{210}Pb concentrations (Figure 9.7b) were however very low. The mean value was just 16 ± Bq kg^{-1}, implying a ^{210}Pb dating horizon of not more than c.30 years. The unsupported ^{210}Pb inventory in the core was 950 Bq m^{-2}. This corresponds to a mean ^{210}Pb supply rate of 30 Bq m^{-2} y^{-1}, around 50% of the estimated atmospheric flux.

Artificial Fallout Radionuclides

The ^{137}Cs activity has a relatively well-resolved peak at a depth of 30.5 ± 4.5 cm (Figure 9.7c) recording the 1963 fallout maximum from the atmospheric testing of nuclear weapons.

Core chronology

Figure 9.8 compares ^{210}Pb dates calculated using the CRS model (Appleby et al. 1978) with the 1963 date determined from the ^{137}Cs record. Use of the CIC model was precluded by the non-monotonic variation in unsupported ^{210}Pb activity.

Table 9.15 Fallout radionuclide concentrations in Barton Broad core BART9

Depth		^{210}Pb						^{137}Cs	
		Total		Unsupported		Supported			
cm	G cm^{-2}	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±	Bq kg^{-1}	±
0.5	0.0	46.9	9.3	21.6	9.5	25.2	2.2	14.6	1.2
5.5	0.8	36.7	8.7	14.6	8.9	22.1	2.1	12.4	1.7
10.5	1.6	37.7	7.3	12.6	7.5	25.1	1.9	12.3	1.4
15.5	2.6	47.2	6.1	24.9	6.3	22.3	1.6	12.0	1.6
20.5	3.6	41.1	7.3	17.3	7.6	23.8	2.1	14.7	1.3
25.5	4.7	25.5	6.9	5.6	7.1	19.9	1.6	12.5	1.3
30.5	5.9	24.8	7.0	-1.9	7.2	26.7	2.0	16.1	1.5
35.5	7.1	39.3	8.0	18.4	8.2	20.9	2.0	13.9	1.5
40.5	8.2	9.1	7.7	-6.2	7.8	15.3	1.4	8.7	1.1
50.5	10.2	11.5	6.9	-9.7	7.1	21.2	1.7	2.9	1.5

Table 9.16 Radiometric chronology of Barton Broad core BART9

(a) Corrected CRS model

Depth		Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	y	±	g cm^{-2} y^{-1}	cm y^{-1}	± (%)
0.0	0.0	2001	0	0			
0.5	0.0	2001	0	1	0.16	1.38	46.3
5.5	0.8	1997	4	2	0.21	1.25	62.8
10.5	1.6	1993	8	3	0.21	0.83	61.5
15.5	2.6	1985	16	4	0.09	0.50	30.7
20.5	3.6	1973	28	6	0.09	0.66	48.0
25.5	4.7	1970	31	6	0.57	1.48	28.3
30.5	5.9	1966	35	5	0.12	0.62	16.8
35.5	7.1	1954	47	11	0.07	0.40	16.8

(b) Assuming a uniform sedimentation rate

Depth		Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	y	±	g cm^{-2} y^{-1}	cm y^{-1}	± (%)
0.0	0.0	2001	0				
0.5	0.0	2001	0	0	0.15	1.04	17.5
5.5	0.8	1996	5	1	0.15	0.93	17.5
10.5	1.6	1990	11	2	0.15	0.80	17.5
15.5	2.6	1983	18	3	0.15	0.74	17.5
20.5	3.6	1976	25	4	0.15	0.72	17.5
25.5	4.7	1969	32	6	0.15	0.65	17.5
30.5	5.9	1961	40	7	0.15	0.62	17.5
35.5	7.1	1953	48	8	0.15	0.63	17.5

The initial ^{210}Pb results place 1963 at a depth of 20.5 cm, significantly above the depth of the ^{137}Cs peak. The discrepancy is almost certainly due to errors in the ^{210}Pb inventory arising from the very low concentrations. Revised ^{210}Pb dates were calculated using the ^{137}Cs date as a reference point (Appleby 2002). The corrected results, also shown in Figure 2 and given in detail in Table 9.16a, suggest significant fluctuations in the net rate of accumulation of sediment during the past 50 years, ranging from ~0.5 cm y^{-1} in the late 1950s and late 1970s to more the 1.3 cm y^{-1} in the late 1960s and late 1990s. Since the validity of the CRS model in this environment is however questionable, Table 9.16b presents an alternative chronology using the mean sedimentation rate of 0.15 ± 0.02 g cm^{-2} y^{-1} determined from the ^{210}Pb and ^{137}Cs records. Differences between the two chronologies are however for the most part fairly small.

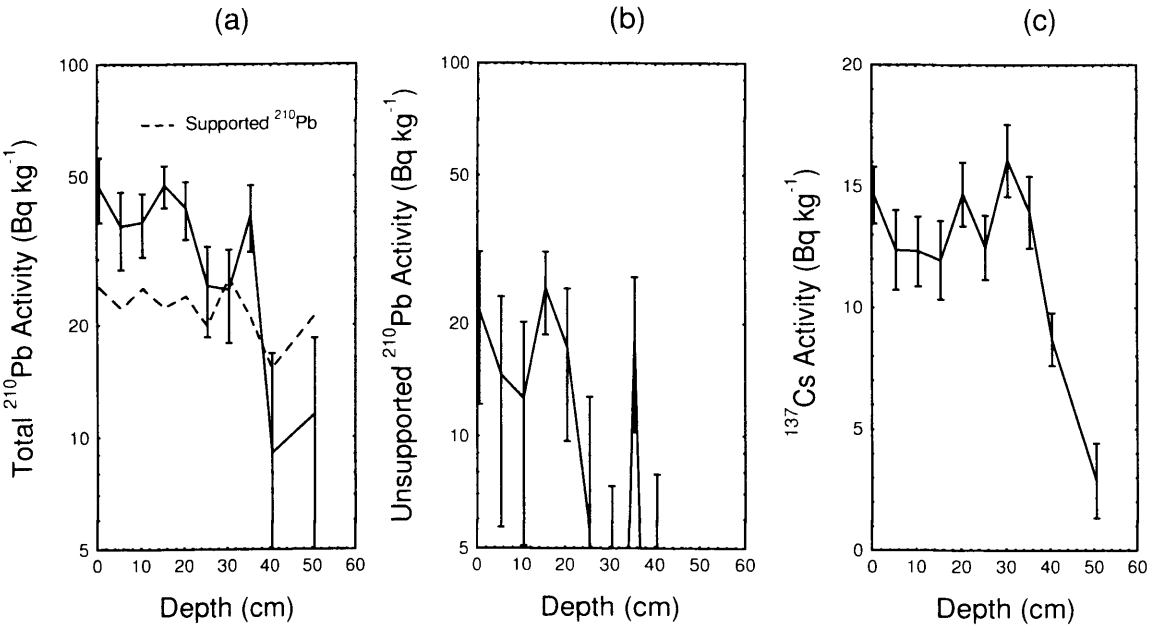

Figure 9.7 Fallout radionuclides in Barton Broad core BART9 showing (a) total and supported ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs concentrations versus depth.

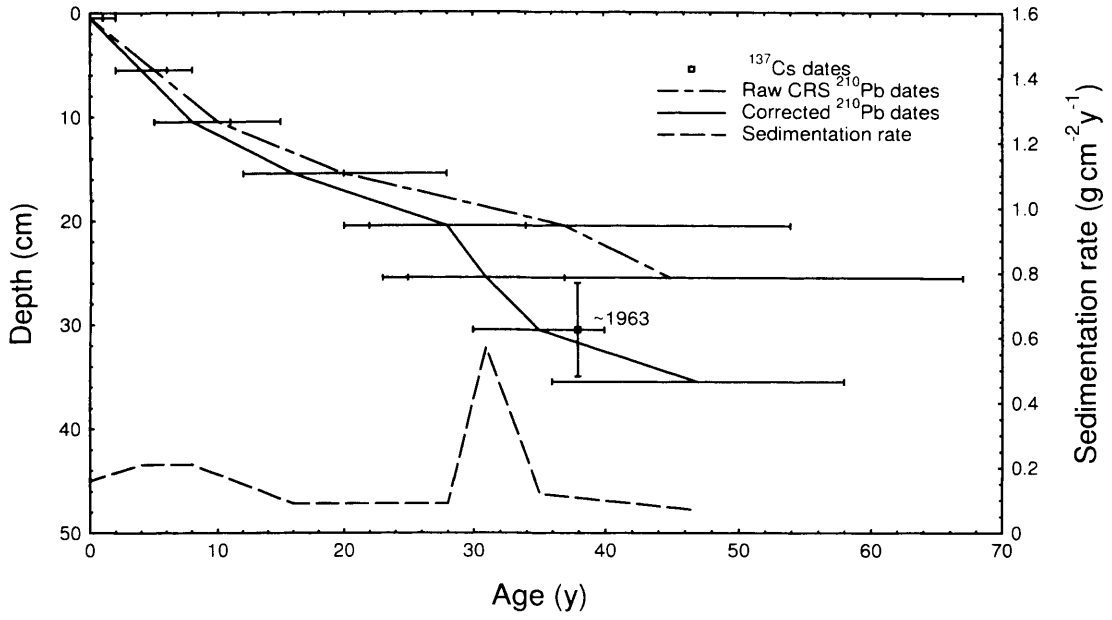

Figure 9.8 Radiometric chronology of Barton Broad core BART9 showing the CRS model ^{210}Pb dates and the 1963 depth determined from the ^{137}Cs stratigraphy. Also shown are corrected CRS model dates calculated using the ^{137}Cs date as a reference point, and the sedimentation rate versus time.

9.4 Organotin surface sediment concentrations

Table 9.17 Organotin surface sediment concentrations

Site Type Name	Organotin concentration ng g-1				
	April 2003		August 2004		
	TBT	DBT	TBT	DBT	MBT
mid-river channel					
d/s Briston	-[a]	-	<5[b]	<4	
d/s Aylsham	-	-	<2	<2	
d/s Coltishall	-	-	<2	<2	
d/s/ Rackheath	-	-	<7	<6	
d/s Belaugh	-	-	<3	<3	
u/s Horstead Mill	<3	<2	<2	<1	
u/s Wroxham Rail Bridge	34	<4	<5	<5	
u/s Wroxham Broad	174	52	157	69	
u/s Decoy Broad	186	71	141	57	126
u/s Ranworth Broad	106	38	86	<6	
u/s South Walsham Broad	-	-	34	22	
River Ant mouth	-	-	124	<3	
broads					
Burntfen Broad	-	-	<20	<17	
Woodbastwick Turfpond	-	-	<24	<20	
Cockshoot Broad	<47	<23	<16	<13	
Upton Broad	-	-	<15	<13	
Pedham Lake	-	-	<17	<15	
Hudson's Bay	-	-	<10	<9	
Salhouse Little Broad	-	-	66	<6	
Hoveton Great Broad	<18	<8	<6	<5	
Decoy Broad	-	-	106	<9	
Ranworth Broad	73	29	165	56	
Wroxham Broad	174	61	135	54	
Salhouse Broad	82	45	118	<5	
Hoveton Little Broad	-	-	<6	<5	
Malthouse Broad	-	-	636	164	
South Walsham Broad	-	-	528	121	
boatyards/marinas					
Connoisseur Boatyard	729	184	1095	246	276
Loynes Boatyard	547	103	978	333	289
Barnes Boatyard	1016	203	569	156	
Landamores Dyke	3288	963	6890 (7376)[c]	965 (913)[c]	614 (297) [c]
Horning Ferry Marina	3319	673	680	141	170

[a] indicates that no sample was collected during the sampling episode

[b] < indicate concentrations below the method quantitation limit

[c] triplicate analysis of the same sample, the result represents the mean and the number in parenthesis indicates the standard deviation

9.5 Sediment core butyltin concentrations

Table 9.18 Concentration of butyltin species (ng g^{-1} D.W.) with depth

a) SALG1

Depth	TBT	DBT	MBT
1-2	110	49	nd
4-5	77	32	"
7-8	259	72	"
10-11	315	98	"
13-14	460	126	"
16-17	405	120	"
19-20	732	196	"
22-23	686	170	"
25-26	748	200	"
28-29	691	164	"
31-32	479	106	"
34-35	320	87	"
37-38	29	9	"
40-41	23	-	"
43-44	14	-	"
50-51	-[a]	-	"
65-66	-	-	"

b) HGBO1

Depth	TBT	DBT	MBT
0-1	-	-	-
3-4	-	-	-
6-7	-	-	-
9-10	87	-	-
12-13	-	-	-
15-16	50	-	-
18-19	88	30	-
21-22	178	48	-
24-25	60	-	-
27-28	-	-	-
30-31	-	-	-
33-34	-	-	-
36-37	-	-	-
39-40	-	-	-
43-44	-	-	-
46-47	-	-	-
49-50	-	-	-

c) HICK1

Depth	TBT	DBT	MBT
1.0-1.5	58	27	-
3.0-3.5	55	31	-
4.5-5.0	59	19	-
6.0-6.5	86	29	-
7.5-8.0	58	24	-
9.0-9.5	64	21	-
10.5-11.0	95	32	-
12.0-12.5	102	32	-
15.0-15.5	-	-	-
18.0-18.5	-	-	-
21.0-21.5	-	-	-
24.0-24.5	-	-	-
27.0-27.5	13	-	-

d) BART9

Depth	TBT	DBT	MBT
0-1	139	98	101
3-4	123	41	25
6-7	112	24	14
9-10	322	53	33
12-13	292	50	22
15-16	184	39	24
18-19	205	40	17
21-22	206	38	27
24-25	4051	271	68
27-28	214	36	25
30-31	378	52	21
33-34	289	42	23
36-37	252	44	19
39-40	194	30	-
42-43	174	34	20
45-46	51	-	-
48-49	46	-	-
51-52	69	-	-

[a] – not detected

9.6 Toxicity endpoints for freshwater organisms <2000 ng l^{-1} dissolved TBT

CAS No.	Scientific Name	Common Name	Endpoint	Effect	Trend	Conc. ng l^{-1}	Test Duration	Exposure Type	Sig.	Sig. Level	Ref.
56359	Scenedesmus quadricauda	Green algae	EC50	GRO	DEC	16	12 d	S	NA	NA	3
56359	Chironomus plumosus	Midge	LC50	MOR	INC	50	96 h	S	NA	NA	3
56359	Biomphalaria glabrata	Snail	EC50	REP	NR	100	14 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	EC80	REP	NR	100	85 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	LC60	MOR	INC	100	85 d	-	NA	NA	1
56359	Tubifex tubifex	Tubificid worm	LC50	MOR	INC	100	96 h	S	NA	NA	3
56359	Tubifex tubifex	Tubificid worm	LC50	MOR	INC	100	96 h	S	NA	NA	3
1461229	Oncorhynchus mykiss	Rainbow trout	LC25	MOR	INC	200	110 d	-	NA	NA	1
56359	Anodonta cygnea	Swan mussel	-	GRO	-	200	8 d	S	-	-	8
56360	Culex pipiens	Mosquito	LC50	MOR	INC	290	24 h	-	NA	NA	1
56359	Lymnaea stagnalis	Great pond snail	-	REP	-	320	33 d	R	-	-	10
56359	Poecilia reticulata	Guppy	-	GRO	-	320	91 d	R	-	-	10
56359	Culex pipiens	Mosquito	LC50	MOR	INC	380	24 h	-	NA	NA	1
56359	Lymnaea stagnalis	Great pond snail	EC50	REP	-	380	33 d	R	NA	NA	10
56359	Scenedesmus quadricauda	Green algae	EC50	POP	DEC	380	12 d	S	NA	NA	3
56359	Daphnia magna	Water flea	LOEC	REP	NR	400	21 d	-	-	SIG	1
56359	Daphnia magna	Water flea	-	GRO	-	560	20 d	R	-	-	10
56359	Culex pipiens	Mosquito	LC90	MOR	INC	690	24 h	-	NA	NA	1
56360	Culex pipiens	Mosquito	LC90	MOR	INC	690	24 h	-	NA	NA	1
1461229	Phoxinus phoxinus	Minnow	-	MOR	-	820	9 d	R	-	-	4
1461229	Phoxinus phoxinus	Minnow	-	MOR	-	840	9 d	R	-	-	5
1461229	Hexagenia sp.	Mayfly	LOEC	GRO	DEC	900	21 d	L	SIG	P<0.05	2
1461229	Hexagenia sp.	Mayfly	IC50	GRO	DEC	920	21 d	L	MULT	NA	2
56359	Biomphalaria glabrata	Snail	EC100	REP	NR	1000	50 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	EC100	REP	NR	1000	85 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	EC90	REP	NR	1000	14 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	LC65	MOR	INC	1000	34 d	-	NA	NA	1
56359	Biomphalaria glabrata	Snail	LC95	MOR	INC	1000	85 d	-	NA	NA	1
1461229	Oncorhynchus mykiss	Rainbow trout	LC90	MOR	INC	1000	110 d	-	NA	NA	1
56359	Daphnia magna	Water flea	-	MOR	-	1000	14 d	R	-	-	10
56359	Daphnia magna	Water flea	-	MOR	-	1000	20 d	R	-	-	10
56359	Daphnia magna	Water flea	-	REP	-	1000	20 d	R	-	-	10

CAS No.	Scientific Name	Common Name	Endpoint	Effect	Trend	Conc. ng l	Test Duration	Exposure Type	Sig.	Sig. Level	Ref.
56359	Lymnaea stagnalis	Great pond snail	-	MOR	-	1000	26 d	R	-	-	10
56359	Lymnaea stagnalis	Great pond snail	-	MOR	-	1000	33 d	R	-	-	10
56359	Poecilia reticulata	Guppy	-	GRO	-	1000	28 d	R	-	-	10
1461229	Oncorhynchus mykiss	Rainbow trout	-	GRO	DEC	1000	110 d	NR	SIG	P<0.05	12
1461229	Oncorhynchus mykiss	Rainbow trout	-	MOR	INC	1000	110 d	NR	-	-	12
1461229	Hexagenia sp.	Mayfly	LC50	MOR	DEC	1200	21 d	L	NA	NA	2
1461229	Hexagenia sp.	Mayfly	LOEC	MOR	DEC	1200	21 d	L	SIG	P<0.05	2
1461229	Daphnia magna	Water flea	-	MOR	NEF	1250	21 d	R	-	-	11
1461229	Daphnia magna	Water flea	-	DVP	CHG	1250	21 d	R	-	-	11
1461229	Daphnia magna	Water flea	-	REP	CHG	1250	21 d	R	-	-	11
56359	Lymnaea stagnalis	Great pond snail	LC50	MOR	-	1500	33 d	R	NA	NA	10
56359	Oncorhynchus mykiss	Rainbow trout	LC50	MOR	-	1520	96 h	F	NA	NA	9
56359	Oncorhynchus mykiss	Rainbow trout	LC50	MOR	-	1610	96 h	F	NA	NA	9
56359	Rana temporaria	Frog	LC50	MOR	-	1650	96 h	S	NA	NA	6
56359	Daphnia magna	Water flea	LC50	MOR	INC	1670	48 h	-	NA	NA	1
56359	Daphnia magna	Water flea	LC50	MOR	-	1670	48 h	S	NA	NA	7
1461229	Hyalella azteca	Scud	LOEC	GRO	DEC	1700	14 d	L	SIG	P<0.05	2
56359	Daphnia magna	Water flea	LC50	MOR	-	1800	20 d	R	NA	NA	10
56359	Daphnia magna	Water flea	-	MOR	-	1800	14 d	R	-	-	10
56359	Daphnia magna	Water flea	-	MOR	-	1800	7 d	R	-	-	10
56359	Daphnia magna	Water flea	-	REP	-	1800	7 d	R	-	-	10
1461229	Hexagenia sp.	Mayfly	-	MOR	INC	1800	21 d	L	-	-	2
56359	Oncorhynchus mykiss	Rainbow trout	LC50	MOR	-	1840	96 h	F	NA	NA	9
56359	Oncorhynchus mykiss	Rainbow trout	-	GRO	DEC	2000	21 d	F	SIG	P<0.01	13

Key for Appendix 9.6

CAS No.	TBT compound name	Effect		Exposure Type		Significance	
56359	Hexabutyldistannoxane	GRO	Growth	F	Flow-through	SIG	Statistically significant result
1461229	Tributylchlorostannane	DVP	Development	S	Static	MULT	Multiple statistic, sig. as some results
56360	(Acetyloxy)tributylstannane	REP	Reproduction	L	Leaching	NA	Not applicable
		POP	Population	P	Pulse		
		MOR	Mortality	R	Renewal		

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9.7 Fossil pigments

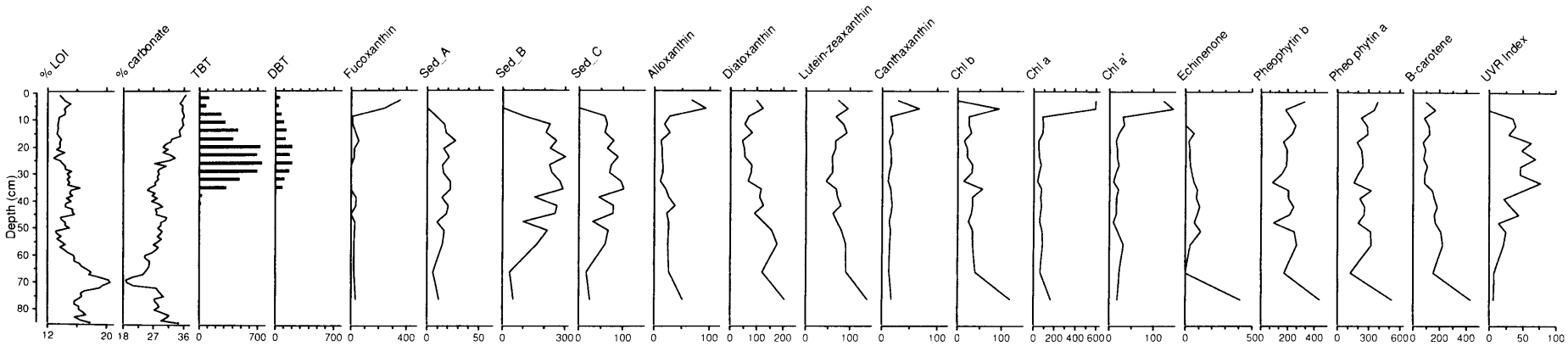

Figure 9.9 Stratigraphy of fossil pigments from core SALG1 (Analysed by Dr. S. McGowan)